Studies on the ammonogenous fungi(Dissertation_全文)

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STUDIES ON THE AMMONOGENOUS FUNGI

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CONTENTS

SCOPE OF THE PRESENT WORK*

In a series of experiments to observe responses of higher fungi to chemical disturbances of forest soil ecosystem, urea was found to bring about the occurrence (formation of reproductive structures on the soil surface)** of a group of fungi and to cause some striking changes in soil properties (Sagara & Hamada, 1965). To clarify the ecological or physiological meanings of these phenomena, the following investigations were carried out.

1) The fungi which occurred after the urea treatment was listed up from various vegetations developed in many parts of Japan and how the urea effect is modified by the region or the type of vegetation was examined.

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** The words "occurrence (or to occur)" and "appearance (or to appear)" are used in this sense. The words "reproductive structures" indicate basidiocarps in Basidiomycetes, ascocarps in Ascomycetes, conidiophores and conidia in Deuteromycetes, and sporangiophores and sporangia in Zygomycetes. Further, the words "to yield (to produce, or to obtain) a species (or a fungus)" mean "to bring about the occurrence of a fungus species".

2) Various chemicals or agents were applied to the soil to find out the substitute for urea and also to elucidate the factors responsible for the urea effect.

3) Natural habitats of the fungi occurred after the treatment with urea or some other ammonia-releasing materials were searched for.

4) Responses of soil and other organisms to the chemical treatments and in the natural habitats were examined to find out the characteristics of the place of occurrence of the fungi in question.

5) Taxonomic positions of these fungi were studied and their habitats mentioned in literature were listed up to compare with the results obtained in the present studies.

The present investigations dealt with the soils of uncultivated lands, especially of forests. Only the reproductive stages are discussed, though I observed on many occasions that a considerable vegetative growth did precede the reproduction (cf p. 110, footnote). Through these studies, I will propose a new ecological grouping of fungi to stimulate further studies.

An outline of the studies was published in a previous paper (Sagara, 1973). Succession (sequential appearance) of the fungi, fruit body production, effects of temperature (or season) and concentration of the chemicals, and some other aspects will be described and discussed in separate papers.

HISTORICAL

Gilbert (1875) observed, after repeated applications of fertilizers to meadow-land for twenty years, a luxuriant occurrence of "Marasmius oreadum" in fairy rings exclusively "on the plot with superphosphate alone" and "on the plot with superphosphate of lime, sulphate of soda and magnesia but without potass for fourteen years". But this was somewhat different from the scope of the present studies (see Part IV for the significance of "repeated application"), and, as shown in Table 1, only a little findings have been reported on the occurrence of fungi after a single application of chemicals to soil.

Effects of the treatment with urea and its related nitrogen compounds on soil have variously been studied, e.g. by Lees & Quastel (1946b), Franz (1956), Cooke (1962), Court, Stephen & Waid (1962), and Roberge & Knowles (1966, 1967). However, the formation of reproductive structures on the surface of soil (i.e. occurrence) after the treatment with such ammonia-releasing chemicals had never been reported before us (Sagara & Hamada, 1965; Sagara, 1973). After the treatment with K_2CO_3 in his studies on fireplace fungi, Petersen (1970b) obtained five (?) of the species which, in the present studies, have been obtained with these ammoniacal

Table 1

Table 1. The fungi reported to have been obtained by the chemical treatment of forest soils

Authors	Agents	Species[a]	Character
Lohwasser, 1953	'Limes'	*Lactarius deliciosus* and 'others'	
Hora, 1958, 1959	$Ca(OH)_2$	*Omphalia maura* *Galactinia praetervisa* *Aleuria lilacina*	'Of burnt ground'
Hora, 1959	'Growmore' $(NH_4)_2SO_4$ Superphosphate	*Lactarius rufus* *Paxillus involutus*	
	$Ca(OH)_2$	*Clitocybe dicolor*	In fairy rings
Franz & Laub, 1959	$CaCO_3$	*Laccaria amethystina*	
Hintikka, 1960	Acetone	*Pholiota carbonaria* *Coprinus boudieri* *Lachnea* sp. (close to *L. melaloma*)	'Pyrophile Arten'
	Butanol	*Coprinus boudieri* *Pholiota carbonaria*	
Sagara & Hamada, 1965	Urea	*Ascobolus denudatus* *Lyophyllum plexipes* f. *typicum*(?) An unidentified discomycete	'Unknown' (='Proteophilous fungi', Sagara, 1973)
Hongo & Sagara, 1967	Urea	*Lyophyllum tesquorum* *Coprinus neolagopus*	(='Proteophilous fungi', Sagara, 1973)
Petersen, 1970a	$CaCO_3$	*Humaria hemisphaeroides* *Peziza praetervisa* *Lamprospora dictydiola* *Octospora* sp.	'Fireplace fungi'
	$Ca(OH)_2$		
Petersen, 1970b	$CaCO_3$	*Ascobolus pusilus* *Fayodia maura* *Humaria hemisphaeroides* *Lamprospora dictydiola* *Octospora* spp. (three) *Peziza endocarpoides* *Peziza praetervisa*	'Fireplace fungi'
		Iodophanus carneus *Omphalia pyxidata* *Peziza granulosa*	'At least common on burnt ground'
	Na_2CO_3	*Lyophyllum tylicolor*	'Of different ecology' (different from fireplace group) (='Proteophilous fungi', Sagara, 1973 except *P. palustris*)
	K_2CO_3	*Peziza* sp. *Ascobolus denudatus* *Coprinus* sp. *Lyophyllum gibberosum* *Lyophyllum tylicolor* *Peziza* sp. *Peziza palustris*	
Korf & Sagara, 1972	Urea Calcium cyanamide	*Humaria velenovskyi*	(='Proteophilous fungi', Sagara, 1973)
Sagara, 1973	Urea, aqua ammonia, and other nitrogenous materials to liberate ammonia; strong alkalis	Twenty-five species to be again mentioned in the present paper	'Proteophilous fungi'
	Calcium cyanamide, $Ca(OH)_2$, $CaCO_3$, Coal-ash, Calcium acetate	Thirteen species (not to be discussed in the present paper and hence not listed)	Fireplace fungi

a. Some are synonymous.

agents and strong alkalis. But he failed to present any ecological or physiological interpretation, describing them as "with different ecology" (Table 1). His question was answered preliminarily in the preceding paper (Sagara, 1973) and more fully in the present paper.

PART I

OCCURRENCE OF FUNGI AFTER THE TREATMENT OF SOIL WITH UREA

Introduction

In a coniferous forest in Kyoto (St. 32; p. 10), a few higher fungi, unknown in Japan then, appeared exclusively on the plots treated with urea, being accompanied with some striking changes in soil properties (Sagara & Hamada, 1965). It was supposed that some more fungi would be obtained by the same or similar response to the urea treatment and that at least some of them might be obtained by treating a small amount of soil with urea in laboratory. In the present study, therefore, effect of urea on the occurrence of fungi was investigated by applying it to soils of various vegetations on the spot or in the laboratory under different seasons or temperatures, respectively. Experimental stands to collect soil samples or to conduct field experiments were chosen so as to cover a wide variety of conditions in Japan. The species which appeared after the urea treatment were recorded to sum up the flora of the fungi responding to the treatment and to clarify its regional difference or relationship between the type of vegetation and the flora of these fungi.

Methods

Experimental stands

The experimental stands were distributed from warm temperate zone to subarctic zone of Japan (Fig. 1). They were numbered from south to north, and their vegetation type, locality, and altitude are listed on p. 9-12. In case some account of the vegetation can be found in the paper by Numata, Miyawaki, & Itoh (1972), the heading number of the paragraph containing the accounts is cited in the parentheses immediately after the designation of vegetation type. These descriptions are followed by the date on which soil sample was collected (C) or on which urea treatment in the field (on the spot) was conducted (F).

(Continued on p. 13)

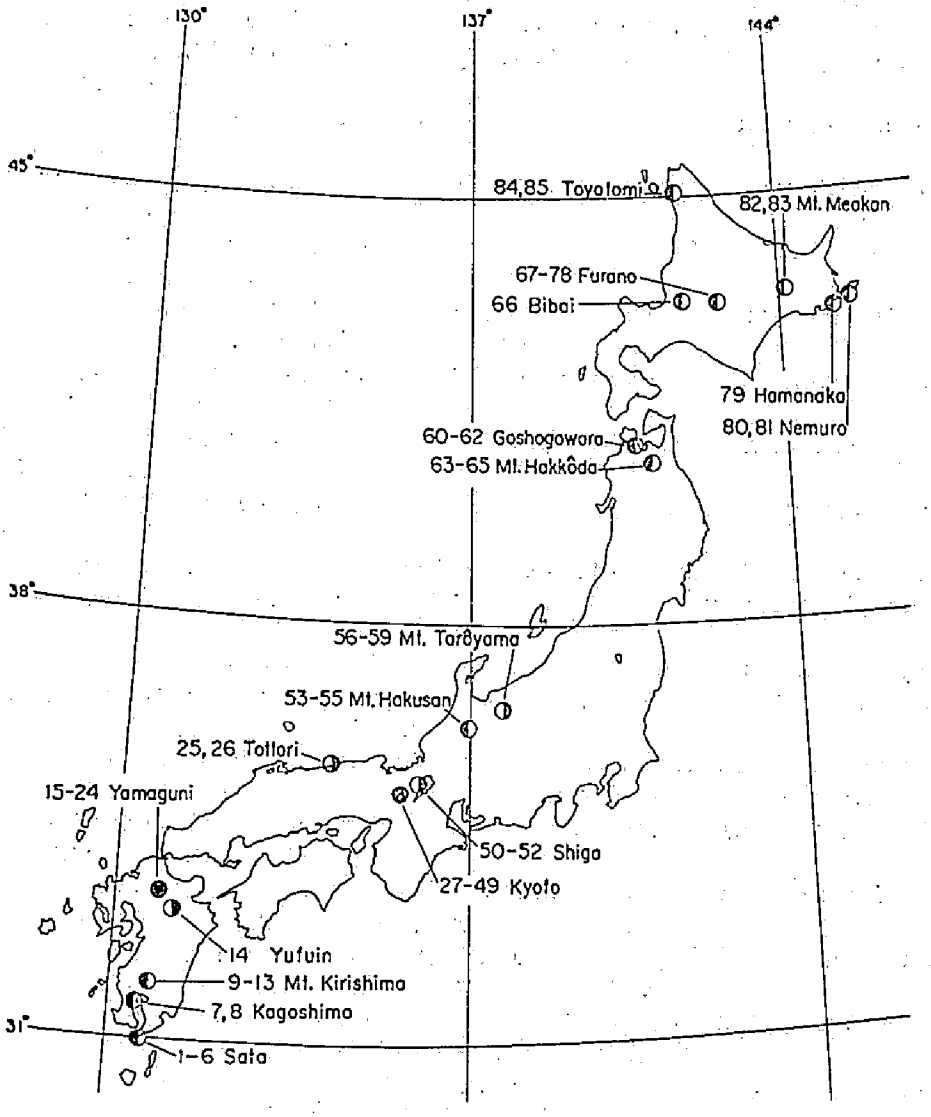

Fig. 1. Location of experimental stands over Japan. The arabic numbers indicate stand numbers. ◐: Stands where soil samples for laboratory experiments were collected. ◑: Stands where field experiments were carried out. ◓: Stands where soil samples were collected and field experiments were carried out.

1. *Ardisia sieblodii-Cinnamomum japonicum-Alpinia intermedia-Polystichopsis pseudoaristata* forest** (1. 1. 1. 2). Sata, Kagoshima; ←80 m. C: 3. iv. 67.
2. *Schefflera octophylla-Ardisia sieboldii-Colysis pothifolia-Piper kazura* forest** (1. 1. 1. 2). Sata, Kagoshima; 70 m. C: 3. iv. 67.
3. Roadside planting of *Livistona subglobosa*. Sata, Kagoshima; 30 m. C: 3. iv. 67***.
4. Roadside planting of *Musa xparadisiaca*. Sata, Kagoshima; 60 m. C: 3. iv. 67***.
5. *Cryptomeria japonica* artificial forest. Sata, Kagoshima; 70 m. C: 3. iv. 67.
6. *Pinus thunbergii* stand on sea-coast (1. 1. 4). Sata, Kagoshima; 10 m. C: 3. iv. 67.
7. *Cinnamomum camphora-Quercus glauca*(?)-*Pleioblastus simoni* forest (?1. 1. 2. 1). Kagaoshima City; 100 m. C: 4. iv. 67.
8. Solitary growth of *Ficus wightiana* on sea-coast. Kagoshima City; near 0 m. C: 4. iv. 67.
9. *Pinus densiflora* forest (1. 2. 1. 2). Mt Kirishima, Kagoshima; 780 m. C: 31. iii. 67.
10. *Chamaecyparis obtusa* artificial forest. Mt. Kirishima, Kagoshima; 780 m. C: 31. iii. 67.
11. *Tsuga sieboldii-Pinus densiflora-Pleioblastus distichus var. nezasa*(?) forest (1. 1. 2. 2). Mt. Kirishima, Kagoshima; 1200 m. C: 1. iv. 67.
12. *Rhododendron kiusianum-Pleioblastus distichus var. nezasa*(?)-*Miscanthus sinensis* wind-blown scrub* (2. 2. 2). Mt. Kirishima, Kagaoshima; 1400 m. C: 1. iv. 67.
13. *Picea polita-Fagus crenata-Pleioblastus distichus var. nezasa*(?) forest (?2. 1. 1). Mt. Kirishima, Kagoshima; 1330 m. C: 1. iv. 67.
14. *Pleioblastus distichus var. nezasa* grassland* (2. 2. 2). Yufuin, Ōita; 950 m. F: 6. ix. 68.
15. *Quercus acutissima* artificial forest*. Yamaguni, Ōita; 220 m. C: 5. iv. 67. F: 21. xi. 67; 20. vii. 68.
16. *Quercus glauca* coppice (1. 2. 1. 1). Yamaguˆni, Ōita; 230 m. C: 5. iv. 67. F: 21.& 23. xi. 67; 20. vii. 68.
17. *Pinus densiflora* artificial stand. Yamaguni, Ōita; 320 m. C: 5. iv. 67. F: 21 & 23. xi. 67; 20. vii. 68.
18. *Quercus serrata* coppice* (?1. 2. 1. 3). Yamaguni, Ōita; 340 m. C: 5. iv. 67. F: 21. xi. 67; 20. vii. 68.
19. *Pinus densiflora* forest (natural; 1. 2. 1. 2). Yamaguni, Ōita; 350 m. F: 21. xi. 67; 20. vii. 68.
20. *Chamaecyparis obstusa* artificial forest. Yamaguni, Ōita; 270 m. C: 6. iv. 67. F: 21. xi. 67; 20. vii. 68.
21. *Cryptomeria japonica* artificial forest. Yamaguni, Oita; 250 m. C: 6. iv. 67. F: 23. xi. 67; 20. vii. 68.
22. *Quercus glauca* coppice (1. 2. 1. 1). Yamaguni, Ōita; 210 m. F: 23. xi. 67; 20. vii. 68.
23. *Phyllostachys heterocycla* f. *pubescens* stand (1. 2. 1. 4). Yamaguni, Ōita; 180 m. C: 6. iv. 67. F: 27. xi. 67; 20. vii. 68.
24. *Fagus crenata* forest* (2. 1. 1). Yamaguni, Ōita; 990 m. C: 6. iv. 67. F: 22. xi. 67; 19. vii. 68.

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* Summergreen. Others are evergreen or mixed.

** After Sako, S.: Bull. Fac. Agr. Kagoshima Univ. No. 13, 205-220 (1963); often regarded as subtropical.

*** The leaves which had been cut down and were decaying on the ground were collected.

25. *Pinus thunbergii* artificial stand on sand dune of sea-coast (dwarfish; the growth very poor and the ground exposed to sun-light). Tottori City; 30 m. F: 20. viii. 69.
26. *Pinus thunbergii* artificial stand on sand dune of sea-coast (rather tall; the growth better than in no. 25 and the ground shaded from sun-light). Tottori City; 35 m. F: 20. viii. 69.
27. *Castanopsis cuspidata* forest (?1. 1. 2. 1). Kyoto City; 120 m. F: 24. ii. 67; 13. viii. 67.
28. *Phyllostachys bambusoides* (bamboo) stand (1. 2. 1. 4). Kyoto City; 95 m. F: 27. ii. 67; 13. viii. 67.
29. *Castanopsis cuspidata* forest (?1. 1. 2. 1). Kyoto City; 150 m. F: 24. ii. 67; 13. viii. 67.
30. *Castanopsis cuspidata* forest (?1. 1. 2. 1). Kyoto City; 150 m. F: 24. ii. 67; 13. viii. 67.
31. *Castanopsis cuspidata* forest (?1. 1. 2. 1). Kyoto City; 140 m. C: 4. x. 67. F: 24. ii. 67; 13. viii. 67.
32. *Pinus densiflora-Chamaecyparis obtusa* forest (1. 2. 1. 2). Kyoto City; 190 m. C: 4. x. 67. F: 19. ii. 67; 13. viii. 67.
33. *Pinus densiflora-Chamaecyparis obtusa* forest (young) (1. 2. 1. 2). Kyoto City; 190 m. F: 20. ii. 67; 13. viii. 67.
34. *Cryptomeria japonica* artificial stand. Kyoto City; 170 m. C: 4. x. 67. F: 25. ii. 67; 13. viii. 67.
35. *Chamaecyparis obtusa* artificial stand. Kyoto City; 150 m. C: 4. x. 67. F: 25. ii. 67; 13. viii. 67.
36. *Quercus acutissima-Q. serrata* stand* (?1. 2. 1. 3). Kyoto City; 140 m. C: 4. x. 67. F: 25. ii. 67; 14. viii. 67.
37. *Cryptomeria japonica* artificial forest. Kyoto City; 220 m. F: 26. ii. 67; 14. viii. 67.
38. *Quercus acutissima* artificial stand*. Kyoto City; 230 m. F: 26. ii. 67; 14. viii. 67.
39. *Quercus acutissima* artificial stand*. Kyoto City; 210 m. F: 26. ii. 67; 14. viii. 67.
40. *Chamaecyparis obtusa* artificial stand. Kyoto City; 180 m. F: 26. ii. 67; 14. viii. 67.
41. *Phyllostachys nigra* f. *henonis* (bamboo) stand (1. 2. 1. 4). Kyoto City; 160 m. C: 11. x. 67. F: 25. ii. 67; 14. viii. 67.
42. *Pinus densiflora* forest (young) (1. 2. 1. 2). Kyoto City; 110 m. F: 27. ii. 67; 14. viii. 67.
43. *Pinus densiflora* forest (1. 2. 1. 2). Kyoto City; 110 m. F: 27. ii. 67; 14. viii. 67.
44. *Castanopsis cuspidata* forest (?1. 1. 2. 1). Kyoto City; 160 m. F: 24. ii. 67; 13. viii. 67.
45. Weed community in the campus of Kyoto University*; 60 m. F: 2. iii. 67; 15. viii. 67.
46. Weed community in the campus of Kyoto University*; 60 m. F: 2. iii. 67; 18. viii. 67.
47. *Cinnamomum* stand in the Botanical Garden, Kyoto University; 70 m. F: 15. viii. 67.
48. *Castanopsis-Pasania-Quercus* stand in the Botanical Garden, Kyoto University; 70 m. F: 15. viii. 67.
49. *Aphananthe-Ulmus* stand in the Botanical Garden, Kyoto University; 70 m. F: 15. viii. 67.
50. *Pinus densiflora* forest (young) (1. 2. 1. 2). Shiga, Shiga; 120 m. F: 25. xi. 66.
51. *Quercus serrata-Q. variabilis* forest* (?1. 2. 1. 3). Shiga, Shiga; 120 m. F: 5. xi. 66.

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* See p. 9.

52. *Fagus crenata* forest* (2. 1. 1). Mt. Hira, Shiga; 1000 m. F: 5. xi. 66.
53. *Fagus crenata* forest* (2. 1. 1). Mt. Hakusan, Ishikawa; 1200 m. C: 17. ix. 67.
54. *Abies mariesii* stand (3. 1). Mt. Hakusan, Ishikawa; 2000 m. C: 17. ix. 67.
55. *Pinus pumila* thicket (4. 1). Mt. Hakusan, Ishikawa; 2400 m. C: 17. ix. 67.
56. *Fagus crenata* forest* (2. 1. 1). Mt. Tarōyama; Toyama; 1400 m. F: 10. viii. 68.
57. *Abies mariesii-Sasa kurilensis* forest (3. 1). Mt. Tarōyama, Toyama; 1750 m. F: 9. viii. 68.
58. Subalpine grassland* (4. 2). Mt. Tarōyama, Toyama; 2040 m. F: 9. viii. 68.
59. *Pinus pumila* thicket (4. 1). Mt. Tarōyama, Toyama; 2340 m. F: 9. viii. 68.
60. *Quercus dentata-Q. mongolica* var. *grosseserrata* forest* (?2. 2. 1. 2). Goshogawara City, Aomori; 140 m. C: 3. ix. 67.
61. *Pinus densiflora* forest (1. 2. 1. 2). Goshogawara City, Aomori; 200 m. C: 3. ix. 67.
62. *Thujopsis dolabrata* var. *hondai* forest. Goshogawara City, Aomori; 180 m. C: 3. ix. 67.
63. *Fagus crenata* forest* (2. 1. 1). Mt. Hakkōda, Aomori; 800 m. C: 4. ix. 67.
64. *Abies mariesii* stand (3. 1). Mt. Hakkōda, Aomori; 1060 m. C: 5. ix. 67.
65. *Pinus pumila* thicket (4. 1). Mt. Hakkōda, Aomori; 1550 m. C: 5. ix. 67.
66. Drained high-moor land (see no. 84 for the living state). Bibai City, Hokkaidō; 18 m. C: 29. viii. 67 (Sample *a* was taken at 13-25 cm deep, and Sample *b* at 50-60 cm deep; both were peat).
67. *Picea abies***** artificial forest. Furano City, Hokkaidō; 240 m. C: 21. viii. 67
68. *Pinus sylvestris*****artificial forest. Furano City, Hokkaidō; 230 m. C: 23. viii. 67.
69. *Pinus strobus***** artificial forest. Furano City, Hokkaidō; 260 m. C: 23. viii. 67.
70. *Larix olgensis* var. *koreana***** artificial forest*. Furano City, Hokkaidō; 400 m. C: 23. viii. 67.
71. *Betula maximowiczii-Sasa senanensis*(?) forest*. Furano City, Hokkaidō; 400 m. C: 23. viii. 67.
72. *Abies sachalinensis-Picea jezoensis* forest (3. 6). Furano City, Hokkaidō; 400 m. C: 23. viii. 67.
73. *Betula ermani-Picea jezoensis-Sasa senanensis*(?) forest (3. 6). Furano City, Hokkaidō; 730 m. C: 21. viii. 67.
74. *Betula ermani-Sasa senanensis*(?) stand. Furano City, Hokkaidō; 930 m. C: 21. viii. 67.
75. *Tilia japonica-Abies sachalinensis-Sasa senanensis*(?) forest (3. 6). Furano City, Hokkaidō; 430 m. C: 22. viii. 67.
76. *Betula ermani-Abies sachalinensis-Picea jezoensis-Sasa senanensis*(?) forest (3. 6). Furano City, Hokkaidō; 600 m. C: 22. viii. 67.

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* See p. 9.
**** Exotic.

77. *Betula ermani-Picea jezoensis-Abies sachalinensis-Sasa kurilensis*(?) forest (3. 6). Furano City, Hokkaidō; 800 m. C: 22. viii. 67.
78. *Pinus pumila-Sasa kurilensis* thicket (4. 1). Furano City, Hokkaidō; 1225 m. C: 22. viii. 67.
79. High moor (2. 1. 6). Hamanaka, Hokkaidō; near 0 m. C: 27. viii. 67 (Dead plant materials at 0-20 cm deep were collected).
80. *Quercus mongolica* var. *grosseserrata-Betula platyphylla* var. *japonica* stand*. Nemuro City, Hokkaidō; 35 m. C: 26. viii. 67.
81. *Abies sachalinensis* forest (3. 6). Nemuro City, Hokkaidō; 45 m. C: 27. viii. 67.
82. *Picea glehnii-Abies sachlinensis* forest (3. 7). Mt. Me-akan, Hokkaidō; 850 m. C: 25. viii. 67.
83. *Pinus pumila* thicket (4. 1). Mt. Me-akan, Hokkaidō; 1350 m. C: 25. viii. 67.
84. High moor (2. 1. 6). Toyotomi, Hokkaidō; 4 m. C: 31. viii. 67 (Dead plant materials at 0-10 cm deep were collected).
85. *Betula platyphylla* var. *japonica-Sasa senanensis*(?) stand*. Toyotomi, Hokkaidō; 5 m. C: 31. viii. 67.

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* See p. 9.

(Continued from p. 7)

Laboratory experiments

Design. a) Soil used: The layer of organic matters accumulated over the mineral soil; in forests it formed O horizon (raw humus layer, organic layer).

b) Amount of soil treated in one container: Wet soil equivalent to 20 g dry soil.

c) Container used: Wide-mouthed glass bottle, 6.5 or 7.5 cm diam (3.2 cm diam at the mouth) and 17.0 cm deep (Pl. 2, A).

d) Temperatures employed: 10 C and 25 C; the former to yield the fungi occurring after winter treatment in the field and the latter those occurring after summer treatment (see p. 17).

e) Amount of urea applied: 0.2 or 0.4 g N in 20 ml solution per bottle, that is, 1 ml of aqueous solution containing 10 or 20 mg N was applied to the soil equivalent to 1 g dry matter.

f) Series of the treatment:

	Temperature (C)	Amount of urea (g N per bottle)
Ser. i	10 (± 1)	0.2
Ser. ii	10 (± 1)	0.4
Ser. iii	25 (± 1)	0.2
Ser. iv	25 (± 1)	0.4

The controls (untreated series) were not prepared because the untreated soils had not yield any fungus in some preliminary experiments.

Collection of soils. Soil samples for the laboratory experiments were collected from the stands located in the Prefectures Kagoshima, Ôita, Kyoto, Ishikawa, Aomori, and Hokkaidô (Fig. 1). In each stand, the organic layer--the soil--was cut out with a edged-tool at several points and packed in a polyethylene bag. The stand number was used as the soil number. The soils were preserved at low temperatures without drying till further treatments.

Procedures before incubation. First, each soil was stirred and mixed well to make it homogeneous. A small portion (10 g) was used for the determination of water content (oven-dry at 105 C). From the rest, soils to weigh 20 g if dried were put in the glass bottles. Through all these procedures, possible cares were taken not to cause mutual contamination of the soils.

Urea solution was applied with a pipet to the soils in the bottles. Each bottle was covered at its mouth with filter paper (Toyo Roshi no. 6) to maintain proper aeration and to minimize the chance of contamination by air. The bottles were then placed in a phytotron (10 C) or in incubators (25 C). Light was provided continuously from fluorescent lamps. The urea application and incubation were conducted on 7 May 1967 in Experiment I which treated the soils from Kagoshima and Ôita, and on 12 Oct. 1967 in Exp. II which treated the soils from other places.

Procedures during incubation. a) Addition of water. In the initial periods following the application of urea, the soils absorbed a large quantity of water in parallel

with the increase in water-holding capacity of soil (see Part IV). The water given in the form of urea solution was not enough to ensure this process, so that distilled water was added at some intervals.

b) Draining. Some time after the addition of water, certain quantity of fluid stagnated at the bottom of each bottle. It was dark reddish-brown, probably containing decomposition products, waste products, etc. It appeared to suppress the growth of fungi and therefore was poured out from the bottle. Care was taken not to destroy the texture of soil and mycelia.

c) Washing. After the initial periods mentioned above, the rate of water absorption and the rate of organic matter decomposition decreased. But it was still necessary to supply water to the soils at least to supplement the loss by natural desiccation and to clean away the undesired materials. For these purposes, the soils were washed as follows: (i) Each bottle was filled with water sterilized by boiling (cool when used); (ii) The soil in the bottle was stirred and kept standing for a short time to absorb the water and to release the undesired materials; (iii) The water was then decanted leaving a small amount of water in the bottle, otherwise a small amount of sterilized water was newly added.

These treatments were more frequently conducted in Ser. iii and iv than in Ser. i and ii, because all processes, biological, chemical, or physical, appeared to proceed more rapidly at higher temperature.

Observation. The treated soils were observed every

day, every second day, or every third day in the early stages, when the changes in fungus flora were rapid, and every seven days in the later stages. Name of the species appeared was recorded, and the matured fruit bodies were picked out from the bottle after the recording. The observation was continued till 23 May 1970 in Exp. I and till 23 Apr. 1969 in Exp. II.

Problem of contamination. The majority of the fungi occurred after these procedures might not originate from contaminants but from their hyphae or spores which had been present in the soils since the time of collection. The following may support this view. The peats (Soils 66a, b) produced no fungus in this experiment (Table 1). When the same peats were inoculated, prior to the urea treatment, with cultured hyphae or spores of several fungi obtained beforehand by the treatment of other soils with urea, at least one of them did occur but, again, not others. This implies that the peats contained some nutrients, enabling the fungi to grow only if urea was added, but that they were devoid of the fungus flora. Thus, contaminations, if any, may not cause serious confusions to the results.

Field experiments

Plots. The field experiments were conducted in the stands located in the Prefectures Ôita, Tottori, Kyoto, Shiga, and Toyama (Fig. 1). In each stand, experimental plots, usually three, each being 0.5 m wide and 1 m long,

were marked out along the slope. Untreated area surrounding the plots was regarded as the control. The plots were labelled with a consequtive number along the date of treatment and the treatment series (see below) throughout the field experiments (Parts I, II).

Treatments. Fertilizer urea (granular form, N 46 %) was spread on the ground surface by hand scattering at the rates of 40, 80, and 160 g N per plot. These are designated as treatment series i, ii, and iii, respectively.

In several stands, another treatment was added: The O horizon was removed and then urea was applied on the A1 or A2 horizon.

In most stands the experiments were commenced at two different seasons, i.e. winter (November-February) and summer (July, August), since I had found that the fungus flora to be obtained was most typically different when treated in these two seasons. The date of the treatment was mentioned in the preceding list of the stands.

Observation. The experimental plots were frequently observed in the early stages so as not to overlook the fungi which appear for short periods. The intervals of observation were prolonged towards the later stages as the changes in fungus flora became slow and their occurrence was usually limited to the so-called "fungus seasons", i.e. early summer and autumn. Name of the species occurred was recorded, and the larger fruit bodies, especially of the basidiomycetes, were picked off after the recording. The observation was continued till autumn of 1969 in Shiga (Sts. 50-52), autumn

of 1971 in Ôita (Sts. 14-24) and Kyoto (Sts. 27-49), summer of 1972 in Tottori (Sts. 25, 26), and till autumn of 1973 in Toyama (Sts. 56-59).

Results and Discussion

The results of the laboratory experiments are shown in Table 2 and those of the field experiments in Table 3. The term "exclusively" (Table 3) is explained by Pl. 1, A. The term "relatively luxuriantly" (Table 3) refers to the cases in which the fruit bodies were more abundant or larger, or both, than in the controls and is explained by Pl. 1, B, C. A few data of the fungus succession are inserted here (Fig. 2), though it will be described and discussed fully in a separate paper. The first two of the three data used in Fig. 2 (Plots 200, 222) were from the experiments not mentioned in the Methods, but they were in principle the same as those obtained in the present experiments. The general feature may be as follows:

Deuteromycetes → Ascomycetes → Basidiomycetes
(→ Deuteromycetes).

The treatments in the laboratory did not yield the larger basidiomycetes which appear in the later stages in the field experiments, but had second deuteromycete phase after the basidiomycete phase.

(Continued on p. 22)

Table 2. The fungi formed reproductive structures on the surface of the soils collected from various vegetations of Japan and treated with urea in laboratory

L, occurred at 10 C (Treatm. ser. i or ii, or both); H, occurred at 25 C (Treatm. ser. iii or iv, or both); LH, occurred both at 10 C and 25 C; (—) A, occurred in the experiments not mentioned in the Methods. Small letters indicate questions remaining on the identifications, and blanks indicate possibilities of occurrence in further experiments.

Soil no.[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	18	20	21	23	24	31	32	34	35	36	41	53	54	55	60	61	62	63	64	65	66[b]	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85
Deuteromycetes																																																								
Amblyosporium botrytis																LH							A	L			L		L		L	L			L	LH	LH		L					L	LH		LH		L		L	L	LH			LH	
Cladorrhinum foecundissimum									H										H		H		A	H	H	H	H				H	H	H	H														H							
Doratomyces putredinis	L	L	L	LH	LH	LH	L	LH		L					L			L	L				A		L	L	H												L	L										L						
Oidiodendron truncatum							L		L	L	L	L	L		L			L						L	L				L		L			L			L	L																		
Stysanus medius	L		L	L		LH							L	L		L				L	L	L	L			L		L				L														LH										L	
Ascomycetes																																																								
Ascobolus denudatus(?)	L	l			H				Ll	LH	Lh	LH	L	L		LH	LH	L	L		LH	LH	LH	LH	LH	LH	l	LH	LH	LH	l	LH	L	L	LH	LH		Lh			Lh			LH		lh	LH	Lh	L	Lh	LH	L	LH	L	Lh
Ascobolus sp. no. 2										LH		H																H				H			H																	H		
Fimaria(?) sp.		L			L	LH		l	L	L			L		L	L	L	L		L							L			L		L																							
Gelatinodiscus sp.									h		h		h										H																																
Melastiza sp.		L																	L																																					
Peziza sp. no. 1											l					L		L					A	L							L																								
Basidiomycetes																																																								
Coprinus lagopus	H	H	H	H		H	H	H							H							h				h	H									H			H		H														
Coprinus narcoticus[c]																							A	L					L						1	L	H																		
Coprinus neolagopus									H	H	h	H		H	H	H	H	H	H	H	H	H	A	H		H	H			H		H	H					H	H		H		H	H	H	H	H	H	H	H	H	H	H	H
Coprinus phlyctidosporus	LH	LH	LH	LH	LH		LH		LH		LH	L	L		L		LH	L		L	LH	L		LL	L	L		L			L	L					L		H	L	LH	L					LH				1		
Coprinus stercorarius																										H																	L		L									
Coprinus sp. no. 2													L			1	L			L		L	L	1	L	L			L	L	L	L		L	L	L			L			L	L			L	1	L		L	L			
Coprinus sp. no. 7																										L				L												LH	L	L	L	L		L						
Coprinus sp. no. 8[c]															1		L			1																																				
Lyophyllum tylicolor									L			LH	LH								L		LH	L	L	L		L		LH					L	LH										LH				LH	L		
Panaeolina(?) sp. no. 3[c]																																													L								

a. Identical with the stand number (p. 9-12).

b. Including the samples *a* and *b* (p. 11).

c. Requiring further data of occurrence for confirmation ("doubtful species", p. 24).

Table 3

Table 3. The fungi formed reproductive structures exclusively or relatively luxuriantly on the grounds in various vegetaions of Japan after treatment with urea

The occurrence in either one or two or all of the Treatment ser. i-iii is presented totally by one letter. W, occurred after the winter treatment; S, occurred after the summer treatment; WS, occurred after both winter and summer treatments; A, occurred in the experiments not mentioned in the Methods. In Sts. 14, 25, 26, 47-49, and 56-59 only the summer treatment was conducted, and in Sts. 50-52 only the winter treatment. Small letters indicate questions remaining on the identifications, and blanks possibilities of occurrence in further experiments.

Stand no.	14	15	16[c]	17[c]	18	19	20	21	22	23	24[c]	25	26	27	28	29	30	31	32[c]	33[c]	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	56	57	58	59
Deuteromycetes																																										
Amblyosporium botrytis	S	W	W	W	W				W	W	W		S	S		S	W	S	S		S	A		W			W		WS	S	S	S						S		S	
Cladorrhinum foecundissimum															S	S			S		S					S					S	S	S	S						
Ascomycetes																																										
Ascobolus denudatus(?)		W			W		W							WS	W		WS	WS	WS	WS	W	W		W		WS	WS	WS		S	W		W	WS	WS	WS		A		A	W	W	W		S	S		S
Ascobolus sp. no 2																	A																									
Chaetomium globosum[d]																																				S							
Fimaria(?) sp.	W	W	W	W	W	W	W	S	W	W	W			W	W	W	W	W	W	A	W	W	W	WS	WS	WS	WS	W	W	W	W	W	W		A	W	W	W				
Gelatinodiscus sp.				S							S		S	S		S	S	S	S	WS	S	S	S	S			S		S		S								S		
Humaria velenovskyi																			WS																	W			S	S		S
Melastiza sp.																															W	W	A						S			
Peziza sp. no. 1	W	W	WS	W	W	W	W	W	W	W			S	W	W	W	W	W	W	A	WS	W		WS		W	W	W	W	A	W	W	W			W		W	S	S		S
Trichophaea gregaria			W																																							
Basidiomycetes																																										
Collybia cookei	WS		W								S																															
Collybia(?) sp.					w														A																							
Coprinus lagopus															S						S													s			S				
Coprinus neolagopus		S				S			S		S			S	S	S	S	S		S		S	S	S		S	S	S	S	S	S	S	S	S	S	A	S	S			
Coprinus phlyctidosporus	S	S		S			S	W	S		s	S		S	S		A			S	S	WS		S	WS	WS				WS	S		S	S	S		S			
Coprinus sp. no. 2	W	W	W	W			W	W	W		W	WS	W	WS	W	W	A	W	W	W	W					w		W	W	WS				W		W		S		S
Coprinus sp. no. 7																										W															
Hebeloma radicosum														WS		WS	WS	WS	A	A	S		WS					WS	W	W												
Hebeloma spoliatum	S	W	W	WS	W			WS		WS		S	W		W	WS	WS	A					WS			WS	W		WS	A						W	W	W	s			
Hebeloma vinosophyllum	S	WS	WS	S	WS			S				S	WS		S	WS	WS	A			S		WS				S		S		WS					W					
Laccaria proxima		S								WS								WS	WS					W				W												S	
Lactarius chrysorheus																		A										W												S	
Lepista subnuda																															W										
Lyophyllum constrictum(?)[a] ..																								W	W		W	W														
Lyophyllum gibberosum														W		W		W	W	W		W								W						W					
Lyophyllum tylicolor	S	W	WS	WS		WS				WS		S	WS		WS	WS	WS	WS	WS	WS	WS	WS				S		WS	WS	WS								W	S	S		S
Panaeolina rhombisperma									S				S																			s	s								
Panaeolina(?) sp. no. 1																			A																						
Rhizopogon rubescens(?)																			W	A																					
Rhodophyllus babingtonii[b, d]																																			A						

Stand no.	14	15	16[c]	17[c]	18	19	20	21	22	23	24[c]	25	26	27	28	29	30	31	32[c]	33[c]	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	56	57	58	59
Deuteromycetes																																										
Amblyosporium botrytis	S	W	W	W	W				W	W	W		S	S		S	W	S	S	S	A			W			W		WS	S	S	S							S		S
Cladorrhinum foecundissimum															S	S			S	S						S				S	S	S	S								
Ascomycetes																																										
Ascobolus denudatus(?)		W			W	W								WS	W		WS	WS	WS	WS	W	W		W		WS	WS	WS		S	W		W	WS	WS	WS		A		A	W	W	W	S	S		S
Ascobolus sp. no 2																	A																									
Chaetomium globosum[d]																																			S							
Fimaria(?) sp.	W	W	W	W	W	W	W	S	W	W	W			W	W	W	W	W	W	A	W	W	W	WS	WS	WS	WS	W	W	W	W	W	W			A	W	W	W			
Gelatinodiscus sp.			S								S		S	S		S	S	S	S	WS	S	S	S	S			S		S	S	S								S		
Humaria velenovskyi																			WS																	W			S	S	S
Melastiza sp.																														W	W	A							S		
Peziza sp. no. 1	W	W	WS	W	W	W	W	W	W	W	W		S	W	W	W	W	W	W	A	WS	W		WS		W	W	W	A	W	W	W				W		W	S	S	S
Trichophaea gregaria			W																																						
Basidiomycetes																																									
Collybia cookei	WS		W								S																														
Collybia(?) sp.						w													A																						
Coprinus lagopus															S								S				s						S								
Coprinus neolagopus			S					S			S			S	S	S	S	S	S		S		S	S	S	S		S	S	S	S	S	S	A	S	S				
Coprinus phlyctidosporus	S	S		S					S	W	S		s	S		S	S	A			S	S	WS		S	WS	WS			WS	S		S	S	S			S		
Coprinus sp. no. 2	W	W	W	W					W	W	W		W	WS	W	WS	W	W	A	W	W	W	W				w		W	W	WS					W		W		S	S
Coprinus sp. no. 7																											W														
Hebeloma radicosum														WS		WS	WS	WS	A	A	S		WS					WS	W	W												
Hebeloma spoliatum	S	W	W	WS	W				WS		WS		S	W		W	WS	WS	A				WS			WS	W		WS	A						W	W	W	s			
Hebeloma vinosophyllum	S	WS	WS	S	WS				S				S	WS		S	WS	WS	A		S		WS				S		S		WS						W				
Laccaria proxima		S									WS								WS	WS						W			W											S	
Lactarius chrysorheus																			A										W											S	
Lepista subnuda																															W										
Lyophyllum constrictum(?)[a]																						W	W			W	W														
Lyophyllum gibberosum														W		W		W	W	W			W							W							W				
Lyophyllum tylicolor	S	W	WS	WS		WS				WS			S	WS		WS	WS	WS	WS	WS	WS	WS	WS				S		WS	WS	WS							W	S	S	S
Panaeolina rhombisperma										S					S																S	S								
Panaeolina(?) sp. no. 1																			A																						
Rhizopogon rubescens(?)																			W	A																					
Rhodophyllus babingtonii[b, d]																																			A						

a. Or *L. leucocephalum* (?).

b. Forma *japonicus*.

c. Including the plots where O horizon was removed prior to the urea application (p. 17).

d. Requiring further data of occurrence for confirmation ("doubtful species", p. 24).

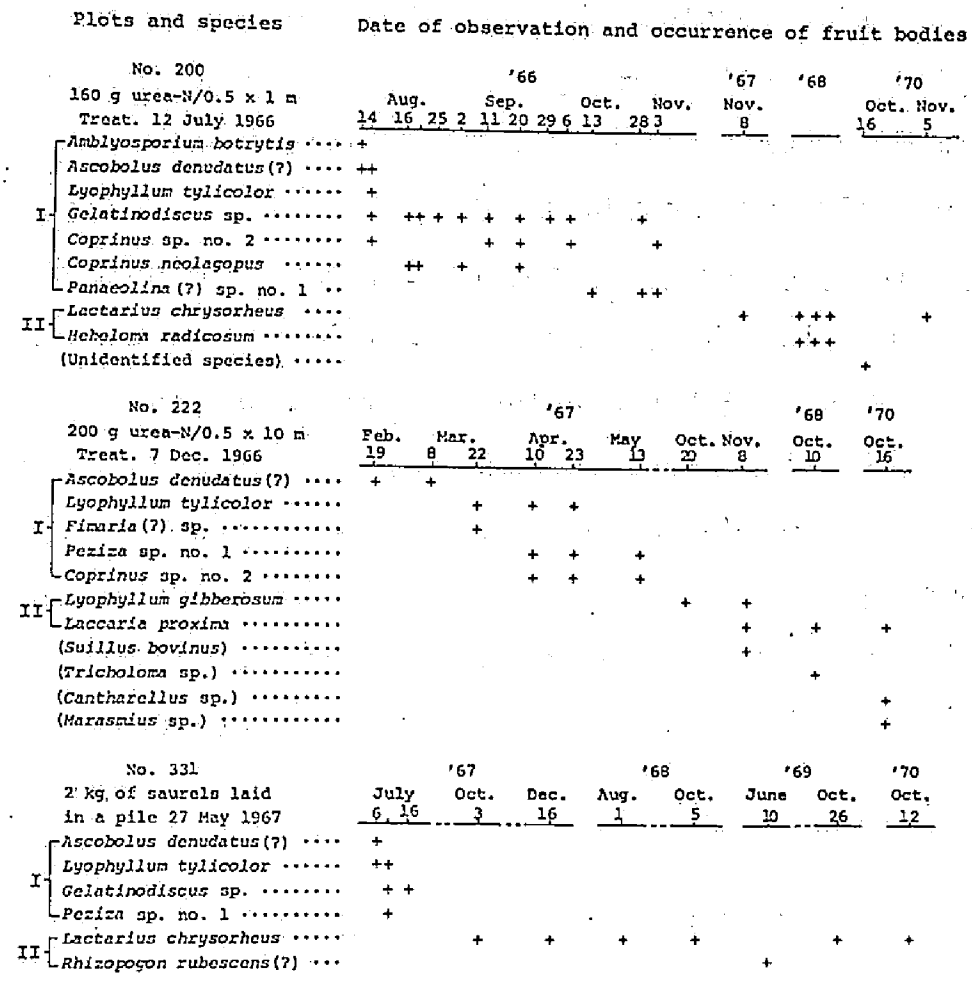

Fig. 2. Examples of the sequential occurrence of fungi on the ground applied with urea or dead bodies of animal in the Pinus-Chamaecyparis forest (St. 32). In Plot 200 urea was incorporated into the O horizon and in Plot 222 urea was spread on the surface (both not mentioned in the Methods, see the text). As for Plot 331 see Part II and Pl. 2, C. The numbers in Greek capital (I, II) indicate the grouping of urea fungi (p. 24). The species in the parentheses were considered to have appeared after a partial recovery of original or normal fungus flora and they are not included in the "urea fungi" (p. 24).

(Continued from p. 18)

Te be added to the list of the species obtained in the field experiments are Cantharellus minor(?) and Rhodophyllus lampropus. In a experiment not mentioned in the Methods, these appeared relatively abundantly on urea plot (and calcium cyanamide plot) in a young Pinus-Chamaecyparis forest near by St. 32 (not mentioned in the list of the stands). And, as shown in Tables 2 and 3, the data obtained in the experiments outside the present studies include some records of occurrence in some fungi which were missed in the present experiments. These mean that, in some places, the species which appear after the urea treatment will increase if further treatments are conducted under wider variety of conditions.

Universality of the phenomena

The occurrence of special fungi and the changes in soil properties after the urea treatment were more or less observed in almost all the stands and soils collected (see Part IV for the changes in soil properties). Since the majority of the identified species have been known in Europe and North America (see Part V), it is presumed that these phenomena will universally be observed at least all over the warm temperate to subarctic regions of the Northern Hemisphere if proper environmental conditions are provided.

Exceptions and their possible causes are as follows.

a) In the laboratory experiment, the peats collected from deep layers of drained high moor (Soils 66a, b) yielded no fungus. This would be due to lack of the fungus flora to

respond to the urea treatment (p. 16): The surface layer of living high more (Soils 79, 84) did yield a few of the fungi in question.

b) In the field experiment, the Pinus thunbergii plantation on sand dune (St. 25) produced no fungus. In this case, severe desiccation owing to the poor growth of the pine (p. 10) and little accumulation of organic matter must have retarded the growth of the fungi.

c) The subalpine grassland (St. 58) also produced no fungus. But, when the soil sample from this stand was treated in the laboratory, some of the fungi in question were obtained (not shown in Table 2). This indicates that these fungi were potentially present in the soil but failed to appear under the natural field conditions.

Definition of "urea fungi"

The number of the species which responded to the urea treatment by developing reproductive structures on the soil surface did not increase unlimitedly when the experiments were extended from Kyoto district to other parts of Japan. The species not obtained in Kyoto but in other places were only two; Trichophaea gregaria (St. 17) and Collybia cookei (Sts. 15, 17, 24). This may mean that the majority of the fungi in question can be obtained within a small district, if the experiments are repeated in a variety of vegetations and under different seasons (the treatment and observation was most frequently carried out in Kyoto district) and that

the assemblage of the species does not differ largely from district to district. Thus, the occurrence-inducing or growth-promoting effect of urea treatment, whether it is a primary or secondary one, seems to be observed in a limited number of fungal species. I propose to put these species into a group under the term urea fungi. At present, thirty-five species are included in this group, excluding the five doubtful species for which further confirmations are needed (Tables 2, 3).

Grouping of urea fungi

A set of suffixes, "-biont", "-philous", "-xenous", and "-phobous", were adopted by Moser (1949) for the fungi of burnt ground and then by Cooke (1957) for the fungi of polluted water and sewage. These are applicable also to the present group. The fungi whose occurrence is restricted to the urea-treated soils may be termed ureobiont species. The others, occurring relatively luxuriantly on the treated soils, may be termed ureophilous species (Table 4, footnote e). The urea fungi comprises these two groups. Fungi to be termed ureoxenous species, which are indifferent to urea treatment and appear by chance on urea plot, were rare. On the other hand, there were many fungi to be termed ureophobous species, in which the occurrence (and hyphal growth too?) was suppressed by the urea treatment; e.g. Russula densifolia in the Castanopsis cuspidata forests of Kyoto (Sts. 30, 31), Lactarius hatsudake in the Pinus thunbergii plantation of Tottori (St. 26), and some species

Table 4. Grouping of urea fungi by their occurrence in laboratory or field experiment

Group I: The fungi obtained in the laboratory, the majority being obtained also in the field

Deuteromycetes

Amblyosporium botrytis
Cladorrhinum foecundissimum
Doratomyces putredinis[a]
Oidiodendron truncatum[a]
Stysanus medius[a]

Ascomycetes

Ascobolus denudatus(?)
Ascobolus sp. no. 2
Chaetomium globosum[b, f]
Fimaria(?) sp.
Gelatinodiscus sp.
Melastiza sp.
Peziza sp. no. 1

Basidiomycetes

Coprinus lagopus
Coprinus narcoticus[f]
Coprinus neolagopus
Coprinus phlyctidosporus
Coprinus stercorarius
Coprinus sp. no. 2
Coprinus sp. no. 7
Coprinus sp. no. 8[a, f]
Lyophyllum tylicolor
Panaeolina rhombisperma[b]
Panaeolina(?) sp. no. 1[b]
Panaeolina(?) sp. no. 3[a, f]

a. Obtained only in the laboratory.
b. Obtained in laboratory experiments not mentioned in the Methods.

Group II: The fungi not obtained in the laboratory but in the field

Ascomycetes

Humaria velenovskyi[c]
Trichophaea gregaria

Basidiomycetes

Cantharellus minor(?)[d, e]
Collybia cookei
Collybia(?) sp.
Hebeloma radicosum
Hebeloma spoliatum
Hebeloma vinosophyllum
Laccaria proxima[c, e]
Lactarius chrysorheus[e]
Lepista subnuda[e]
Lyophyllum constrictum or *L. leucocephalum*(?)
Lyophyllum gibberosum
Rhizopogon rubescens(?)[e]
Rhodophyllus babingtonii f. *japonicus*[f]
Rhodophyllus lampropus[d, e]

c. But obtained in a laboratory experiment using a larger amount of soil (4 kg in dry wt.).
d. Obtained in a stand not mentioned in the Methods (see p. 22)
e. "Ureophilous" (p. 24). Others are all "ureobiont".
f. Requiring further confirmation to decide as urea fungus ("doubtful species", p. 24).

of the genera Marasmius and Mycena in the Pinus-Chamaecyparis forest of Kyoto (St. 32). Generally speaking, no fungus seemed unaffected by the urea treatment.

The urea fungi can be divided into two groups in another way (Table 4): one occurs in the laboratory experiment (Group I) and another does not (Group II). The characters of the fungi leading to this grouping seem to be correlated with some other features. Generally in the field experiments, the fungi of Group I appeared earlier but lasted for shorter periods than those of Group II in the succession. The fruit bodies of the former are generally smaller and more perishable than those of the latter. All of the former are ureobiont whereas some of the latter are ureophilous. Physiological characters may also be related to this grouping (cf p. 110, footnote).

The differences between the results in the laboratory experiments and those in the field experiments would be attributed, at least to certain extent, to the facts that, in the field, the treated soils were open to the penetration of all kinds of organisms living in the surrounding soils, and that the amount of soil used in the laboratory experiments was rather small.

Regional differences

Repetition of experiment may increase the mark + in Tables 2 and 3 (see p. 22) and will make the difference in the species composition obscure. The regional differences mentioned below, therefore, should be confirmed by further

studies. For the discussion of the results, some unpublished data will be taken into account.

Amblyosporium botrytis. Not obtained from the soils of Kagoshima (Table 2).

Cladorrhinum foecundissimum. Not recorded in Ôita (Table 3): The time of observation might not be adequate as it appears for a short period.

Fimaria(?) sp. Not obtained from the soils of northern Japan (Mt. Hakkôda and Hokkaidô; Table 2).

Peziza sp. no. 1. Not obtained from the soils of northern Japan (Mt. Hakkôda and Hokkaidô) and those of Kagoshima (except Soil 11) (Table 2).

Coprinus neolagopus. Not obtained at higher altitude in Toyama (Sts. 56-59; Table 3). This is plausible as, in the laboratory experiments, this species could appear only when the soils were placed under higher temperatures (20-30 C), at least during an initial period, but see p. 31.

Coprinus sp. no. 2. Not obtained from the soils of Kagoshima except Soil 11 (Table 2).

Coprinus narcoticus. Not obtained from the soils of Kagoshima and Ôita (Table 2).

Collybia cookei. Obtained only in Yamaguni, Ôita (Table 3). The substratum of this fungus was decaying fruit bodies, probably, of the urea fungi which had occurred previously and not been picked off. Some other urea fungi appearing towards later stages also might grow on dead mycelia of the urea fungi grown in the early stages. Namely, it is possible that some of the urea fungi are fungicolous.

This is the reason for including this species in the group of urea fungi despite the fact that it did not appear on the soil itself.

Hebeloma radicosum. Not obtained in Ôita and Toyama (Table 3).

Lyophyllum constrictum or L. leucocephalum(?). Obtained only in a limited area of Kyoto (Table 3).

Lyophyllum gibberosum. Not obtained in Ôita (Table 3): Temperature at the time of treatment (November) might slightly be inadequate for its occurrence as this species appears exclusively after winter treatment in Kyoto.

Relationship with the type of vegetation

The field experiment was rather intensively carried out in Kyoto, so that this problem can be discussed more thoroughly than the preceding one.

Characterization by the flora of urea fungi. The forests of Cryptomeria, Chamaecyparis, and Phyllostachys (bamboo) in Kyoto and Ôita produced very few or none of the fungi of Group II (Table 3). The Fagus forests in Shiga and Ôita (and Toyama also?) yielded two species in common, Hebeloma spoliatum and Laccaria proxima (Table 3). The Pinus densiflora forests at lower altitudes in Kyoto (Sts. 32, 42) and the Pinus pumila thicket at higher altitude in Toyama (St. 59) produced two species in common, Laccaria proxima and Lactarius chrysorheus (Table 3). These results may be interpretted in another form as: Laccaria proxima was obtained after the urea treatment only in the forests of

Pinaceae and deciduous species of Fagaceae; Lactarius chrysorheus was obtained after the urea treatment only in the forests or thicket of Pinaceae. Thus, the same type of vegetations produce the same urea fungi, even if they are located far away from each other.

Intervention of natural fungus flora in the urea-to-fungus reaction system. The fungi of Group II occurred relatively frequently or restrictedly in the forests of Pinus, Quercus, Castanopsis, and Fagus but not in those of Cryptomeria, Chamaecyparis, and Phyllostachys (see the preceding paragraph). This reminds us our empirical knowledge that the general mushroom flora under natural conditions is relatively poor in the latter forests. For example, in Kyoto and Shiga, Lactarius chrysorheus was not rare in pine forests but was seldom met in other forests. After the urea treatment too, this fungus did not appear in the latters. That is, the occurrence of this fungus by the urea treatment was consistent with its natural distribution. ← Some other urea fungi (ureophilous ones) showed the same tendency. The difference in the natural flora seems, to certain extent, to be persistent against urea treatment.

Needlessness of mycorrhiza formation. The species of the genera Pinus, Abies, Quercus, Castanopsis, and Fagus rather form ectomycorrhias, whereas those of Cryptomeria, Chamaecyparis, and Phyllostachys endomycorrhizas. The above-mentioned tendencies that the flora of terrestrial fleshy fungi is richer in the forests of former trees and that the

urea-to-fungus reaction system is intervened by the natural fungus flora ←— are considered to be related to the type of mycorrhiza formation. Some of the urea fungi are likely to form mycorrhia or to live on in rhizosphere under natural conditions (cf Part III). I observed, however, that they did not necessarily require plant roots when they grew on urea plot.

Level land and hill. From the results of the field experiments in Kyoto, a discrepancy in the flora of urea fungi was observed between the vegetations of level land (dwelling area of man, Sts. 45-49) and those of hills (Sts. 27, 29-40, 42-44). The former vegetaions did not yield Gelatinodiscus sp., Coprinus sp. no. 2, Hebeloma radicosum, Hebeloma spoliatum, Hebeloma vinosophyllum, Lyophyllum gibberosum, and Lyophyllum tylicolor, which were popular in the latter vegetations (Table 3). On the contrary, the formers yielded Melastiza sp., Lepista subnuda, and Panaeolina (?) sp. no. 2, which were not obtained in the latters (but see p. 32 for Melastiza sp.). As is actually located in a transition between the level land and the hilly place, the bamboo stands (Sts. 28, 41; see also 23) seems to come to an intermediate position, yielding Coprinus sp. no. 2 and Panaeolina (?) sp. no. 2 which were characteristic to the hill and to the level land, respectively, but not the other fungi mentioned above.

It is reminded in this connection that the general mushroom flora under natural conditions is also different between hills and level lands.

Age of a stand. In a young Pinus-Chamaecyparis stand developing in a close neighborhood of St. 32 (twenty years old; not mentioned in the list of the stands), the mother rock being the same, Cantharellus minor(?) and Rhodophyllus lampropus occurred in appreciable quantities on urea plot (see p. 22). But they did not so in St. 32 (about eighty years old). The age of the stand might have had a meaning for this.

Notes on some species. The followings may be added to the above and should be confirmed in further studies.

Cladorrhinum foecundissimum. Not obtained from the vegetations of higher altitudes (Tables 2, 3). This may coincides with the fact that, in the laboratory experiments, this species could be obtained only when the soils were placed under higher temperatures (20-30 C) at least during an initial period following the treatment.

Oidiodendron truncatum. Obtained rather frequently from the soils of the coniferous forests than from those of other forests (Table 2).

Coprinus lagopus. Obtained from the soils of the forests developing at lower altitudes rather than those at higher altitudes (Tables 2, 3). This is plausible because this fungus had the same habit of occurrence as Cladorrhinum foecundissimum (see above).

Coprinus neolagopus. Obtained from the soils of higher altitudes (Table 2). This is strange because, as is in Cladorrhinum foecundissimum and Coprinus lagopus, this fungus required higher temperatures at least during an initial

period for its occurrence: There should be little chance for this fungus to appear under the field condition (see p. 27).

Melastiza sp. Characteristic for the level land in Kyoto (p. 30) but obtained in Fagus forest in Toyama (St. 56; Table 2).

Summary

The soils (organic layer) of the vegetations representive of the warm temperate to subarctic climates of Japan were treated with urea. The amounts of urea applied were, in the laboratory experiments, 0.2 and 0.4 g N in 20 ml solution per wet soil equivalent to 20 g dry soil or, in the field experiments, 40, 80, and 160 g N per 0.5 x 1 m plot. As a result, sequential formation of reproductive structures of special fungi was observed on the surface of almost all the soils studied. Number of the species appeared was counted 35 in total, of which 29 species were restricted to- and 6 species were relatively luxuriant on the urea-treated soils. They are termed "urea fungi".

The same type of forests at distant places yielded generally the same urea fungi. Some differences in the flora of urea fungi were found between the forests of Cryptomeria and Chamaecyparis and those of Pinaceae and Fagaceae, both developing on hilly places, and between the vegetations of level land and those of hill.

PART II

EFFECTS OF SOME OTHER AGENTS ON THE OCCURRENCE OF FUNGI

Introduction

Application of urea exerts diverse effects upon soil, serving as nitrogen source, alkalizing the soil, killing soil organisms, and stimulating organic matter decomposition. To elucidate the essential substance or factor required for the occurrence of the "urea fungi" (p. 23), effects of various agents on fungus flora were examined.

Methods

The agents used

The chemicals and other agents used in the present experiments may be grouped as follows.

Standard (control). Urea.

Group 1. Aqua ammonia and calcium cyanamide (calcium cyanamide-N takes the form of urea on the way of decomposition).

Group 2. Salts of weak acids and ammonium hydroxide (basic NH_4^+-N, cf Group 7).

Group 3. Dead animal body, and plant material with high protein content.

Group 4. Final products of nitrogen metabolism in animals.

Group 5. Proteins, peptone, and amino acids.

Group 6. Amines.

Group 7. Salts of strong acids and ammonium hydroxide.

Group 8. Nitrites.

Group 9. Nitrates.

Group 10. Miscellaneous nitrogen compounds having similar structure or radical as urea, ammonia, or amine.

Group 11. Nitrogen-free compounds: Carbohydrates, oils, and lipoid (the last contains a very small amount of nitrogen).

Group 12. Nitrogen-free compounds: Carboxylic acids, alcohols, phenols, aldehydes, and mercaptan (by-products of protein decomposition).

Group 13. Alkalis.

Group 14. Salts of strong acids and potassium hydroxide or sodium hydroxide (non-basic K^+ or Na^+ ions).

Group 15. Inorganic acids.

Group 16. Agents or treatments to kill organisms.

Group 17. Organic solvents.

Field experiments

The experiments were carried out in the Pinus densiflora

-Chamaecyparis obtusa forest in Kyoto (St. 32, p. 10). Details are shown in Table 5. The agents were dressed on the surface of 0.5 x 1 m or 0.5 x 10 m plots marked out on the slope (cf Part I). The methods of observation were nearly the same as those described in Part I. In case only one plot could be prepared for one agent, a high rate was adopted since it had been found with the ammoniacal agents and the alkalis that heavy rates were rather successful to detect the fruiting of fungi.

Laboratory experiments

The methods were fundamentally the same as those described in Part I. The soil samples were collected from the O horizon of the Pinus-Chamaecyparis forest (St. 32) in which the field experiments were carried out. Details of the treatments are shown in Tables 6 and 7. The unglazed pot of one series were rowed on a wooden shelf within a large water-proof case. The shelf was set so as to leave some gap between the pots and the bottom of the case where the drained water stagnated. The case was covered with transparent sheet of vinyl polymers, leaving an opening at lateral sides for proper aeration and humidity.

(Continued on p. 43)

Table 5. Treatments and periods of observation in field experiments

Agents	Plot no.	Amounts (for 0.5 x 1 m)	Periods A[a]	Periods B[b]
Urea	120-122[c]	10, 20, 40 g N	5. iv. 66	15. vii. 70
	339-341	40, 80, 160 g N	10. vi. 67	6. x. 73
	567-569	40, 80, 160 g N	20. i. 68	16. vii. 72
	709-711	40, 80, 160 g N	4. vi. 70	2. x. 73
	735	150 g N	2. v. 71	18. x. 73
	772-776	100, 500, 1000, 2000, 4000 g N	15. ii. 72	30. x. 73
	777	140 g N	15. ii. 72	30. x. 73
	820	160 g N	17. viii. 72	30. x. 73
Group 1[d]				
Aqua ammonia	345-347	40, 80, 160 g N in 1 l	10. iv. 67	6. x. 73
	564-566	40, 80, 160 g N in 1 l	20. i. 68	16. vii. 72
	778	1 kg of 25 % soln. (205 g N)	15. ii. 72	30. x. 73
	869	950 g of 28 % soln.(130 g N)	17. viii. 72	30. x. 73
Calcium cyanamide	123-125[c]	10, 20, 40 g N	5. iv. 66	14. x. 73
	342-344	40, 80, 160 g N	10. vi. 67	6. x. 73
	573-575	40, 80, 160 g N	20. i. 68	2. x. 73
Group 2				
$(NH_4)_2CO_3$	779	500 g(130 g N)	15. ii. 72	30. x. 73
Ammonium formate	850	1 kg(222 g N)	17. viii. 72	30. x. 73
Ammonium acetate	348-350	40, 80, 160 g N	10. vi. 67	6. x. 73
	685-687	40, 80, 160 g N	5. ii. 69	2. x. 73
	780	450 g(82 g N)	15. ii. 72	30. x. 73
	854	1 kg(181 g N)	17. viii. 72	30. x. 73
Ammonium oxalate	858	1 kg(197 g N)	17. viii. 72	30. x. 73
Group 3				
Saurel	331,332[e]	2 kg(12-13 fishes)	27. v. 67	6. x. 73
Mackerel	871,873[f]	3.5 kg(4 fishes)	18. viii. 72	30. x. 73
	872[f]	4.3 kg(5 fishes)	18. viii. 72	30. x. 73
Tōfu(curds from soybean)	520,522[e]	10 pieces(98 g proteins)	25. xii. 67	12. x. 70
Group 4				
Urea, ammonia		See above		
Uric acid	736	150 g N	2. v. 71	18. x. 73
Hippuric acid	737	150 g N	2. v. 71	18. x. 73
	831	2 kg(156 g N)	17. viii. 72	30. x. 73

a. Date of treatment. *b*. Date of final or latest observation.
c. 0.5 x 10 m. *d*. See the text for the grouping of agents.
e. Not 0.5 x 1 m; the fishes were placed in a pile.
f. Not 0.5 x 1 m; the fishes were laid side by side.

Table 5 (continued)

Group 5				
Albumin, from eggs	821	1 kg	17. viii. 72	30. x. 73
Zein	822	1 kg	17. viii. 72	30. x. 73
Casein	823	1 kg	17. viii. 72	30. x. 73
Peptone	740	150 g N	2. v. 71	18. x. 73
	824	1 kg(130 g N)	17. viii. 72	30. x. 73
Sodium glutamate	739	150 g N	2. v. 71	18. x. 73
	829	1 kg(136 g N)	17. viii. 72	30. x. 73
L-Glutamic acid	827	1.5 kg(95 g N)	17. viii. 72	30. x. 73
L-Arginine	828	500 g(160 g N)	17. viii. 72	30. x. 73
L-Cystine	826	1 kg(175 g N)	17. viii. 72	30. x. 73
Group 6				
Ethylenediamine	833	500 g(230 g N)	17. viii. 72	30. x. 73
Trimethylamine	834	2 kg of ca. 30 % soln.(142 g N)	17. viii. 72	30. x. 73
Group 7				
$(NH_4)_2SO_4$	126-128[c]	10, 20, 40 g N	5. iv. 66	20. x. 70
	680,681	160, 320 g N	12. xii. 68	6. x. 73
NH_4NO_3	129-131	10, 20, 40 g total N	6. iv. 66	20. x. 70
	682,683	160, 320 g NH_4^+-N	12. xii. 68	6. x. 73
NH_4Cl	712-714	40, 80, 160 g N	4. vi. 70	2. x. 73
Group 8				
$NaNO_2$	715-717	40, 80, 160 g N	4. vi. 70	2. x. 73
Amyl nitrite	859	500 g(60 g N)	17. viii. 72	30. x. 73
Group 9				
$NaNO_3$	718-720	40, 80, 160 g N	4. vi. 70	2. x. 73
KNO_3	783	750 g(104 g N)	15. ii. 72	30. x. 73
$Ca(NO_3)_2 \cdot 4H_2O$	784	500 g(59 g N)	15. ii. 72	30. x. 73
NH_4NO_3	See Group 7			
KNO_3 + KOH (cf. KNO_3, KOH)	722-724	40, 80, 160 g N + 1 l KOH soln.[g]	4. vi. 70 + 12. vi. 70[g]	2. x. 73
	742-744	40, 80, 160 g N + 250 g KOH	2. v. 71	2. x. 73
Group 10				
Urethane	738	150 g N	2. v. 71	18. x. 73
Thiourea	782	500 g(184 g N)	15. ii. 72	30. x. 73
Hydrazine hydrate (N_2H_2 80 %)	787	500 g(350 g N)	15. ii. 72	30. x. 73
Hydroxylamine hydrochloride	788	500 g(101 g N)	15. ii. 72	30. x. 73
Sulfamic acid	789	1 kg(144 g N)	15. ii. 72	30. x. 73
Formamide	843	500 g(155 g N)	17. viii. 72	30. x. 73
Acetamide	844	500 g(119 g N)	17. viii. 72	30. x. 73
Acetonitrile	845	500 g(171 g N)	17. viii. 72	30. x. 73
Aniline	860	1 kg(150 g N)	17. viii. 72	30. x. 73
Nitrobenzene	790	1 kg(114 g N)	15. ii. 72	30. x. 73

c. See p. 36.

g. 0.5 l of 0.5 N soln. was applied to the upper half and 0.5 l of 1 N soln. to the lower half of each plot on 12 July 1970.

Table 5 (continued)

Group 11				
Starch, soluble	791	1 kg	16. ii. 72	30. x. 73
D(+)-Sucrose	875	1 kg	4. ix. 72	30. x. 73
D(+)-Sucrose	792	1 kg	16. ii. 72	30. x. 73
	876	1 kg	4. ix. 72	30. x. 73
D(+)-Glucose, anhydrous	793	1 kg	16. ii. 72	30. x. 73
	877	1 kg	4. ix. 72	30. x. 73
Whale oil	794[h]	1 kg	16. ii. 72	30. x. 73
	878[h]	1 kg	4. ix. 72	30. x. 73
Olive oil	795[h]	1 kg	16. ii. 72	30. x. 73
	879[h]	1 kg	4. ix. 72	30. x. 73
Lecithin, from soybeans	796	1 kg (N 1.76 %)	16. ii. 72	30. x. 73
Group 12				
Formic acid	847	500 g of 90 % soln.	17. viii. 72	30. x. 73
Acetic acid	806	1.5 l of 75 % soln.	16. ii. 72	30. x. 73
	851	500 g (glacial)	17. viii. 72	30. x. 73
n-Butyric acid	837	500 g	17. viii. 72	30. x. 73
Pyruvic acid	834	500 g	17. viii. 72	30. x. 73
Oxalic acid	810	1 kg	16. ii. 72	30. x. 73
	855	500 g	17. viii. 72	30. x. 73
Succinic acid	799	1 kg	16. ii. 72	30. x. 73
Fumaric acid	798	1 kg	16. ii. 72	30. x. 73
DL-Malic acid	839	500 g	17. viii. 72	30. x. 73
Benzoic acid	830	1 kg	17. viii. 72	30. x. 73
Acetone	See Group 17			
Ethanol	543	99.9 %, 2 l	18. i. 68	2. x. 73
	544	70 %, 2 l	18. i. 68	2. x. 73
	545	70 %, 2 l, incorporated	18. i. 68	2. x. 73
	749	70 %, 1 l	3. v. 71	2. x. 73
	750	99.9 %, 1 l	3. v. 71	2. x. 73
	765	70 %, 1 l, incorporated	3. v. 71	2. x. 73
	766	99.9 %, 1 l, incorporated	3. v. 71	2. x. 73
Methanol	808	1 kg	16. ii. 72	30. x. 73
	880	1 kg	4. ix. 72	30. x. 73
n-Amyl alcohol	800	1 kg	16. ii. 72	30. x. 73
iso-Amyl alcohol	801	1 kg	16. ii. 72	30. x. 73
n-Butyl alcohol	802	1 kg	16. ii. 72	30. x. 73
iso-Butyl alcohol	803	1 kg	16. ii. 72	30. x. 73
n-Propyl alcohol	804	1 kg	16. ii. 72	30. x. 73
iso-Propyl alcohol	805	1 kg	16. ii. 72	30. x. 73
Glycerine	861	500 g	17. viii. 72	30. x. 73
Phenol	835	550 g of 90 % soln.	17. viii. 72	30. x. 73
Cresol	755,756	1 l of 5, 10 vol % emulsion with water	3. v. 72	2. x. 73
	836	350 g	17. viii. 72	30. x. 73

h. 0.5 x 0.5 m.

Table 5 (continued)

Formaldehyde	761,762	1 l of 3.7, 7.4 % soln.[i]	3. v. 71	2. x. 73
	841	500 g of 37 % soln.[j]	17. viii. 72	30. x. 73
Acetaldehyde	842	500 g of 80 % soln.	17. viii. 72	30. x. 73
Methyl mercaptane	832	500 g of ca. 5 % soln.	17. viii. 72	30. x. 73
Group 13				
NaOH	785,786	250, 500 g	15. ii. 72	30. x. 73
	868	500 g	17. viii. 72	30. x. 73
KOH	351-353	0.5 l of 1, 2, 4 N soln.	10. vi. 67	6. x. 73
	560-562	1 l of 1, 2, 4 N soln.	20. i. 68	14. x. 72
	721	g (p. 36)	12. vi. 70	2. x. 73
	729	500 g	4. vi. 70	2. x. 73
	741	250 g	2. v. 71	18. x. 73
Potassium formate	849	1 kg	17. viii. 72	30. x. 73
Potassium acetate	853	1 kg	17. viii. 72	30. x. 73
Potassium oxalate	857	1 kg	17. viii. 72	30. x. 73
MgO	726	1 kg	4. vi. 70	2. x. 73
$Mg(OH)_2$	728	1 kg	4. vi. 70	2. x. 73
CaO	725	1 kg	4. vi. 70	2. x. 73
$Ca(OH)_2$	141-143[c]	43, 86[k], 172 g	9. iv. 66	14. x. 73
	546-548	305, 610, 1220 g	18. i. 68	14. x. 72
	727	1 kg	4. vi. 70	2. x. 73
$CaCO_3$	144-146[c]	50, 100, 200 g	9. iv. 66	16. x. 71
Calcium formate	848	1 kg	17. viii. 72	30. x. 73
Calcium acetate	852	1 kg	17. viii. 72	30. x. 73
Calcium oxalate	856	1 kg	17. viii. 72	30. x. 73
Sodium borate	747,748	0.5, 1 l of 0.01 M soln.	3. v. 71	18. x. 73
Group 14				
K_2SO_4	7-9[c]	10.2, 20.4, 40.8 g	22. vi. 65	14. vii. 66
	15, 18, 21[c]	10.2, 20.4, 40.8 g	23. vi. 65	3. i. 66
$NaNO_3$	See Group 9			
KNO_3	See Group 9			
Group 15				
H_2SO_4	354-356	0.5 l of 1, 2, 4 N soln.	10. vi. 67	6. x. 73
	867	500 g (min 95 %)	17. viii. 72	30. x. 73
HNO_3	357-359	0.5 l of 1, 2, 4 N soln.	10. vi. 67	6. x. 73
	866	500 g (69-71 %)	17. viii. 72	30. x. 73
HCl	865	500 g (min 35 %)	17. viii. 72	30. x. 73
H_3PO_4	555-557	1 l of 0.5, 1, 2 M soln.	18. i. 68	16. vii. 72

c. See p. 36.
i. Containing 0.6-0.8 % methanol.
j. Containing 6-8 % methanol.
k. Nearly the same rate as that adopted by Hora (1958).

Table 5 (continued)

Group 16				
$CuSO_4 \cdot 5H_2O$	751,752	250, 500 g	3. v. 71	2. x. 73
$Ca(ClO)_2$	753,754	250, 500 g	3. v. 71	2. x. 73
$NaClO_3$	763,764	250, 500 g	3. v. 71	2. x. 73
Propylene oxide	757,758	250, 500 g	3. v. 71	2. x. 73
	767,768	250, 500 g, incorporated	3. v. 71	2. x. 73
Paraformaldehyde	759,760	250, 500 g	3. v. 71	2. x. 73
Some other poisons	See Group 12.			
Burning (bonfire)[1]	326,327	3 h	8. iii. 67	6. x. 73
	328,329	2 h	16. iii. 67	6. x. 73
	513,514	3.5, 3 h	20. xii. 67	6. x. 73
	583	2.5 h	31. i. 68	6. x. 73
	633,634	2.5 h	1. viii. 68	6. x. 73
	817,818	2.5 h	24. ii. 72	30. x. 73
Autoclaving	558[m]	2 kg/cm^2, 40 min	19. i. 68	14. x. 72
	559[n]	2 kg/cm^2, 40 min	19. i. 68	14. x. 72
	812[o]	1.2 kg/cm^2, 20 min	24. ii. 72	30. x. 73
Bathing[p]	811	60 C, 2-4 h[q]	24. ii. 72	30. x. 73
Steaming in a autoclave	813	100 C, 5 min	24. ii. 72	30. x. 73
Oven-drying[r]	814	105 C, 4 h[s]	24. ii. 72	30. x. 73
	815	130-140 C, 3 h[t]	24. ii. 72	30. x. 73
Dry-distilling[u]	816	4-6 h[v]	24. ii. 72	30. x. 73
Group 17				
Acetone	809	1 kg	16. ii. 72	30. x. 73
	881	1 kg	4. ix. 72	30. x. 73
Ethyl ether	862	2 kg	17. viii. 72	30. x. 73
Benzene	863	2 kg	17. viii. 72	30. x. 73
Xylene	864	2 kg	17. viii. 72	30. x. 73
Alcohols	See Group 12			

l. Different amount of dead wood and twigs were burnt at various intensities over various areas (not 0.5 x 1 m but around or below 0.5 m^2). Consequently, the duration of burning in hours are of little significance.

m. The soil of O horizon was collected from 0.5 x 0.5 m, treated in the laboratory, and returned to the original place.

n. The soil of O horizon and mineral layer was collected from 0.3 x 0.3 x 0.2(depth) m, treated in the laboratory, and returned to the original place.

o. Plot nos. 811-816 were all 0.5 x 1 m, and the soil of O horizon was treated in the laboratory and returned to the original place.

p. The soil was packed in tins and placed in a bath.

q. Temperature of the inner parts of each tins were lower than 40 C at the end of heating.

r. The soil was packed in wire gauze baskets and dried in electric ovens.

s. Temperature of the inner parts of each baskets must have been lower than 100 C even at the end of heating.

t. Temperature of the inner parts of the baskets were 65-70 C at the end of heating.

u. After the suggestion by Hintikka (1960). The soil was packed in tins and placed on the flames of gas.

v. Little ashes were produced.

Table 6. Treatments in laboratory experiments

Agents	Amounts (per wet soil equivalent to 1 g dry soil)	Exp. no. (Table 7)
Control	No application of the agents	9 b, d
Urea	2.5, 5, 10, 20, 40 mg N in 0.67 ml[a] water	9 a-d
	10, 20 mg N; 1 ml water added	40 a, b
Group 1[b]		
Aqua ammonia	2.5, 5, 10, 20, 40 mg N in 0.67 ml	9 a, c
Calcium cyanamide	2.5, 5, 10, 20, 40 mg N, incorporated	9 b, d
Group 2		
Ammonium acetate	2.5, 5, 10, 20, 40 mg N in 0.67 ml water	9 a, c
Group 4		
Uric acid	10, 20 mg N; 1 ml water added	40 a, b
Hippuric acid	10, 20 mg N; 1 ml water added	40 a, b
Group 5		
Peptone	10, 20 mg N; 1 ml water added	40 a, b
Sodium glutamate	10, 20 mg N; 1 ml water added	40 a, b
Group 7		
$(NH_4)_2SO_4$	2.5, 5, 10, 20, 40 mg N in 0.67 ml water	9 a, c
NH_4Cl	2.5, 5, 10, 20, 40 mg N in 0.67 ml water	9 a, c
$(NH_4)_2SO_4$ + KOH	After 101 days in Exp. 9a, 0.67 ml of 0.5 N KOH soln. was added to each soil	9' a
	After 52 days in Exp. 9c, 0.67 ml of 0.5 N KOH soln. was added to each soil	9' b
NH_4Cl + KOH	The same as in $(NH_4)_2SO_4$ + KOH	9' a, b
Group 9		
$NaNO_3$	2.5, 5, 10, 20, 40 mg N in 0.67 ml water	9 b, d
KNO_3	2.5, 5, 10, 20, 40 mg N in 0.67 ml water	9 b, d
$Ca(NO_3)_2 H_2O$	2.5, 5, 10, 40 mg N in 0.67 ml water	9 b, d
Group 13		
NaOH	0.67 ml of 0.25, 0.5, 1, 2, 4 N soln.	9 a, c
	0.67 ml of 0.1, 0.25, 0.5, 1 N soln.	12 a, b
KOH	0.67 ml of 0.25, 0.5, 1, 2, 4 N soln.	9 a, c
	0.67 ml of 0.1, 0.25, 0.5, 1 N soln.	12 a, b
$Mg(OH)_2$	4.9, 9.8, 19.5, 38.9, 77.8 mg; 0.67 ml water added	9 a, c
$Ca(OH)_2$	6.2, 12.4, 24.7, 49.4, 98.7 mg; 0.67 ml water added	9 a, c
	2.5, 6.2, 12.4, 24.7 mg; 0.67 ml water added	12 b, c

a. = $2/3^{ml}_{\wedge}$ dissolved in 10 or 16.7 ml water and then applied to the wet soil equivalent to 15 or 25 g dry soil, respectively (see Table 7).

b. See the text for the grouping of agents.

Table 7. Conditions employed in the laboratory experiments

Exp. no.	Ser.	Sort of container	Soil per container[a]	Temp (C)	Light sourses	Date of soil collection	Date of application & incubation	Date of final observation
9	a	Unglazed pot[b]	15 g	18.5-22	Sun[c]	11. v. 66	14. v. 66	26. viii. 66
	b			19-22	Sun[c] and glow-lamps[d]			26. viii. 66
	c			25-28	Sun[c]			5. vii. 66
	d			25.5-28	Sun[c] and glow-lamps[d]			5. vii. 66
9'	a	Additional treatment to 9a		9-11	Fluorescent lamps[e]		26. viii. 66	20. ii. 67
	b	Additional treatment to 9c					5. vii. 66	3. xi. 66
12	a	Unglazed pot[b]	15 g	19-22				19. viii. 66
	b	Unglazed pot[b]	15 g	25.5-28	Sun[c] and glow-lamps[d]	31. v. 66	1. vi. 66	19. viii. 66
	c	Unglazed pot[f]	25 g	19-22				20. ii. 67
40	a	Glass bottle[g]	20 g	9-11	Fluorescent lamps[e]	26. iv. 71	5. v. 71	23. x. 72
	b			24-25				

a. In dry weight: the portion to be used in the experiment was not dried (see Part I).
b. 9 cm diam at the mouth, 5 cm diam at the bottom, and 7 cm deep.
c. But under the glass roof of a phytotron and mostly in the shade within it.
d. During night.
e. Continuous illumination.
f. 11.5 cm diam at the mouth, 6.5 cm diam at the bottom, and 9 cm deep.
g. The same as those used in Part I.

(Continued from p. 35)

Results and Discussion

The results are shown in Tables 8 and 9. See also Fig. 2 and Pls. 1-3. The calcium compounds (Group 13) and burning (Group 16) produced many pyrophilous (fireplace) fungi (Sagara, 1973) but they are not presented and discussed here. An identified deuteromycete was obtained after the treatment with olive oil and whale oil (Group 11), but this is excluded from the present discussion because it has not been obtained after the treatment with the nitrogenous materials and because the purity of the oils has become the question. (This fungus was mis-identified as "an yeast" in my previous paper [Sagara, 1973].) Trichoderma viride(?) appeared after almost all kind of treatment in summer but this too is excluded from the present discussion because of the obscurity of its character.

Remarks and discussions on each group of the agents are as follows.

Groups 1-6 (NH_3-releasing agents). Aqua ammonia was almost equivalent to urea in its effects on the fungus flora. The term "urea fungi" may better be replaced by the term "ammonia fungi". This will be discussed in the General Discussion. It is likely that other agents of these groups except L-cystine produce the bulk of the urea fungi. All these effective agents have the nature to cause alkaline conditions of soil, either in their own forms* or by the

(Continued on p. 46)

* These, e.g. amines, too will eventually turn to ammonia.

Table 8. *aecyparis* forest in Kyoto

Frequ experiments.

Blank: nc ion remaining on the

	KOH	Potassium formate	Potassium acetate	Potassium oxalate	$Ca(OH)_2$	Some other alkalis	K_2SO_4, (and KNO_3, $NaNO_3$) −14	H_2SO_4, HNO_3, HCl, H_3PO_4 −15	Burning (bonfire) −16	Some other killings	Organic solvents −17
Zygomy											
Mucor spp.	−		+		−		−	−			−
Rhopalomyc	−	−	−	−	−	−	−	−	−	−	−
Deuter											
Amblyospor	−	−	−	−	−	−	−	−	−	−	−
Cladorrhin	−	−	−	−	−	−	−	−	−	−	−
Penicilliu	−	−	−	−	−	−	−	−	−	−	−
Ascomy											
Ascobolus	+	+					−	−	−	−	−
Ascobolus							−	−	−	−	−

Table 8. The fungi formed reproductive structures exclusively or relatively luxuriantly on the ground of *Pinus-Chamaecyparis* forest in Kyoto after treatment with urea and some other agents

Frequency of treatment not equal for the agents. +: occurrence confirmed. -: not occurred and with no prospect of occurrence in further experiments. Blank: not occurred but with a larger or smaller prospect of occurrence in further experiments. ?: occurrence needing confirmation, or question remaining on the identification. A: occurred in the experiments not mentioned in the Methods.

Agents \ Group no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Urea as the standard	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aqua ammonia	"																
Calcium cyananide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$(NH_4)_2CO_3$																	
Ammonium formate		+															
Ammonium acetate																	
Ammonium oxalate																	
Saurel (sea-fish)			+														
Mackerel (sea-fish)			+														
Tōfu (bean-curds)																	
Uric acid				+													
Hippuric acid																	
Albumin																	
Zein																	
Casein					+												
Peptone					-												
Sodium glutamate																	
L-Glutamic acid																	
L-Arginine																	
L-Cystine						-											
Ethylenediamine																	
Trimethylamine																	
$(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl							-										
$NaNO_2$ (cf NaOH, KOH)								-									
Amyl nitrite								-									
$NaNO_3$, KNO_3, $Ca(NO_3)_2$									-								
KNO_3 + KOH (cf KOH)									-								
Formamide									-								
Aniline									-								
Miscellaneous nitrogens									-	-							
Carbohydrates									-		-						
Oils, Lipoid									-								
Carboxylic acids									-								
Ethanol									-								
n-, *iso-*Amyl alcchol									-								
*n-*Butyl alcohol									-			-					
Some other alcohols									-								
Phenol, Cresol									-								
Aldehydes									-								
Methyl mercaptane									-								
NaOH									-								
KOH									-								
Potassium formate									-				+				
Potassium acetate									-								
Potassium oxalate									-								
$Ca(OH)_2$									-								
Some other alkalis									-								
K_2SO_4 (and KNO_3, $NaNO_3$)									-					-			
H_2SO_4, HNO_3, HCl, H_3PO_4									-						-		
Burning (bonfire)																-	
Some other killings																	
Organic solvents									-								-
Zygomycetes																	
Mucor spp.[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhopalomyces strangulatus	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Deuteromycetes																	
Amblyosporium botrytis ...	+	+				+	-		+	+		+	-	+	-	-	-
Cladorrhinum foecundissimum	+						-						-	+	-	-	-
Penicillium lividum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascomycetes																	
Ascobolus denudatus(?)	+	+	+	+	+	+	+	+	?	+	+	+	-	+	-	-	-
Ascobolus sp. no. 2			+		+	+	+		+	+	+		-	?	-	-	-
Byssonectria aggregata ...	-	-	-		+								-		-	-	-
Fimaria(?) sp.	+	+	+										-		-	-	-
Gelatinodiscus sp.	+	+	+	+	+	+	+	+		+	+	+	+	+	-	+	-
Humaria velenovskyi	+								+			+	-	+	-	-	-
Iodophanus carneus		+											-		-	-	-
Peziza sp. no. 1	+	+	+	+	+	+	+					+	-	+	-	-	-
Scutellinia scutellata ...							+						-		-	-	-
Basidiomycetes																	
Collybia(?) sp.	+		+													+	-
Coprinus neolagopus	+					+							-				
Coprinus phlyctidosporus	A		A										-				
Coprinus sp. no. 2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-
Hebeloma radicosum	+	+	+										-				
Hebeloma spoliatum	+	+		+	+	+							-				
Hebeloma vinosophyllum ...	+	+											-				

[a] Zygomycetes

Group no.		1		2				3			4		5								6		7	8		9		10			11		12								13							14	15	16		17
Agents	Urea as the standard	Aqua ammonia	Calcium cyanamide	$(NH_4)_2CO_3$	Ammonium formate	Ammonium acetate	Ammonium oxalate	Saurel (sea-fish)	Mackerel (sea-fish)	Tōfu (bean-curds)	Uric acid	Hippuric acid	Albumin	Zein	Casein	Peptone	Sodium glutamate	L-Glutamic acid	L-Arginine	L-Cystine	Ethylenediamine	Trimethylamine	$(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl	$NaNO_2$ (cf NaOH, KOH)	Amyl nitrite	$NaNO_3$, KNO_3, $Ca(NO_3)_2$	KNO_3 + KOH (cf KOH)	Formamide	Aniline	Miscellaneous nitrogens	Carbohydrates	Oils, Lipoid	Carboxylic acids	Ethanol	*n*-, *iso*-Amyl alcchol	*n*-Butyl alcohol	Some other alcohols	Phenol, Cresol	Aldehydes	Methyl mercaptane	NaOH	KOH	Potassium formate	Potassium acetate	Potassium oxalate	$Ca(OH)_2$	Some other alkalis	K_2SO_4 (and KNO_3, $NaNO_3$)	H_2SO_4, HNO_3, HCl, H_3PO_4	Burning (bonfire)	Some other killings	Organic solvents
Zygomycetes																																																				
Mucor spp.[a]	-	-	-			+			+							+	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		+		-		-	-			-
Rhopalomyces strangulatus	-	-	=	=	=	=	=		+		=	=		=			=	=		=	=	=	=	=	=	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deuteromycetes																																																				
Amblyosporium botrytis	+	+									+	-			+	+			+	-	+		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cladorrhinum foecundissimum	+											-								-	+		-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium lividum	-	-	-	-	-	-	-	-	-	-	-	+	-		-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascomycetes																																																				
Ascobolus denudatus(?)	+	+	+	+		+	+	+	+	+	?		+		+	+	+		+	-	+		-		-	+	+			-	-	-	-	-	-	-	-	-	-	-		+	+					-	-	-	-	-
Ascobolus sp. no. 2				+				+	+		+		+			+			+	-		?	-		-	-				-	-	-	-	-	-	-	-	-	-	-								-	-	-	-	-
Byssonectria aggregata	-	-	-			+														-			-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fimaria(?) sp.	+	+	+																	-			-		-	-				-	-	-	-	-	-	-	-	-	-	-		+						-	-	-	-	-
Gelatinodiscus sp.	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	+	+		-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+			-	-	-	-	-
Humaria velenovskyi	+										+					+			+	-	+	-	-		-	-				-	-	-	-	-	-	-	-	-	-	-						-	-	-	-	-	-	-
Iodophanus carneus			+																	-			-		-	-				-	-	-	-	-	-	-	-	-	-	-								-	-	-	-	-
Peziza sp. no. 1	+	+	+	+		+		+		+						+				-	+		-		-	-	+			-	-	-	-	-	-	-	-	-	-	-		+				+		-	-	-	-	-
Scutellinia scutellata									+											-			-		-	-				-	-	-	-	-	-	-	-	-	-	-						-	-	-	-	-	-	-
Basidiomycetes																																																				
Collybia(?) sp.	+		+																						-	-				-	-	-														+		-	-	-		-
Coprinus neolagopus	+										+				+					-			-		-	-	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coprinus phlyctidosporus	A		A												+					-			-		-	-	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-
Coprinus sp. no. 2	+	+	+	+		+	+	+		+	+		+	+	+	+	+	+	+	-	+	+	-		-	-				-	-	-	-	-	-	-	-	-	-	-	+					-	-	-	-	-	-	-
Hebeloma radicosum	+	+	+													+	+		+	-			-		-	-				-	-	-	-	-	-	-	-	-	-	-						-	-	-	-	-	-	-
Hebeloma spoliatum	+	+		+		+		+								+	?		+	-			-		-	-				-	-	-	-	-	-	-	-	-	-	-						-	-	-	-	-	-	-
Hebeloma vinosophyllum	+	+										+				+	+			-			-		-	-				-	-	-	-	-	-	-	-	-	-	-		+			+	-	-	-	-	-	-	-
Laccaria proxima	+	+	+	+	+	+			+				+			+	+		+		+	+		+	-	-					-	-		?	+						+	+	+			+		-	-	+		
Lactarius chrysorheus	+	+				+		+	+	+			+		+	+							?		-	-					-	-		?	?													-	-	+		
Lyophyllum gibberosum	+	+	+	+		+														-			-		-	-				-	-	-	-	-	-	-	-	-	-	-						-	-	-	-		-	-
Lyophyllum tylicolor	+	+	+	+	+	+	+	+		+	+		+	+	+	+	+	+	+	-	+	+	-		-	-	+	+		-	-	-	-	-	-	-	-	-	-	-		?		+		-	-	-	-	-	-	-
Panaeolina(?) sp. no. 1	+										+									-			-		-	-	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizopogon rubescens(?)	+	+				+		+	+							+							?		-	-				-	-				?	?												-	-	+		

a. Sp. no. 8 with potassium acetate, no. 10 with ammonium acetate, and nos. 11-13 with mackerel; identity between the specimens not yet clarified.

Table 9

Table 9. The fungi formed reproductive structures on the soil collected from *Pinus-Chamaecyparis* forest in Kyoto and treated with urea and some other agents in laboratory

See Table 8 for explanation of symbols.

Agents —Group no.	Urea as the standard	Aqua ammonia /1\	Calcium cyanamide	Ammonium acetate -2	Uric acid /4\	Hippuric acid	Polypeptone /5\	Sodium glutamate	$(NH_4)_2SO_4$, NH_4Cl -7	$NaNO_3$, KNO_3, $Ca(NO_3)_2$ -9	NaOH,	KOH	$Mg(OH)_2$	$Ca(OH)_2$ 13	$(NH_4)_2SO_4$ + KOH	NH_4Cl + KOH
Zygomycetes																
Mucor spp.[a]	-	-	-	+			+	-	-	-	-	-	-	-	-	-
Deuteromycetes																
Amblyosporium botrytis ...	+					-			-	-	-	-	-	-		
Cladorrhinum foecundissimum	+	+				-			-	-	-	-	-	-		
Doratomyces putredinis ...	+							+	-	-	-	-	-	-		
Penicillium lividum	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Stysanus medius	A															
Ascomycetes																
Ascobolus denudatus(?) ...	+	+	+	+	?		+	+	-	-	+	+		?	+	+
Ascobolus sp. no. 2					+		?		-	-						
Gelatinodiscus sp.	+	+	+	+	+	+	+		-	-	+	+	?		+	+
Peziza sp. no. 1	+	+		+					-	-					+	+
Basidiomycetes																
Collybia(?) sp.														+		
Coprinus narcoticus	A								-	-						
Coprinus neolagopus	+	+	+						-	-	-	-	-	-		
Coprinus sp. no. 2	+				+	+	+	+	-	-			-	-	+	+
Lyophyllum tylicolor	+	+		+					-	-			-	-	+	+

a. Sp. no. 1 with ammonium acetate, and no. 2 with peptone; identity between the specimens not yet clarified.

liberation of ammonia produced by their decomposition (cf Part IV). Zein, L-glutamic acid, and hippuric acid were very slowly decomposed on the forest floor and less effective. Carbon-to-nitrogen ratio might influence these aspects. L-cystine was scarcely decomposed and remained in the forms of crystals even fourteen months after the treatment (Plot 826): it did not yield any fungus.

The species not obtained after the treatment with urea but with other agents of these groups are: Mucor spp. with ammonium acetate, mackerel, and potassium acetate; Rhopalomyces strangulatus with mackerel; Penicillium lividum with hippuric acid; Byssonectria aggregata with ammonium acetate; Iodophanus carneus with calcium cyanamide; Scutellinia scutellata with mackerel. Some more species were obtained after the treatment with dead animal bodies, proteins, and peptone, though they are not yet confirmed or identified. These results may mean that the nitrogenous materials more complex than urea produce some flora in addition to the urea fungi. At the same time, the possibility that these complex materials fail to yield some of the urea fungi can not be denied: It is not easy to confirm this.

Since the additional species mentioned above were not obtained after the treatment with nitrogen-free materials (see below), they are integrated with the urea fungi under the general term ammonogenous fungi ("fungi produced by ammonia"). (This group was formerly termed "proteophilous fungi" [Sagara, 1973], but this term is now abandoned

because it can not adequately represent the characteristics of the group, cf General Discussion). Its definition will be given in the General Discussion.

Group 7 (non-basic NH_4^+-N). Lactarius chrysorheus was recorded after the treatment with NH_4NO_3, and Rhizopogon rubescens(?) with $(NH_4)_2SO_4$ and NH_4NO_3 at their higher rates (Plots 680, 682). These are "ureophilous" species (see Part I). But it was not clear whether or not these fungi appeared as a sequence of the treatments. This point will again be discussed later. It can at least be said that the agents of this group will never yield the "ureobiont" species (see Part I). They however turned as effective as urea when KOH was added after the application of the salts (Table 9). These results show that the acid radicals composing the salts are not harmful but that an alkaline condition is necessary for the occurrence of the urea fungi (or ammonogenous fungi).

Group 8 (NO_2^--N). Effect of $NaNO_2$ was similar to that of NaOH or KOH: It would be safe to consider that $NaNO_2$ was equivalent to NaOH in its effect on the fungus flora. Consequently, this is discussed under the Group 13. Amyl nitrite did not produce any fungus.

Group 9 (NO_3^--N). No particular fungus was obtained. Some urea fungi were obtained by the addition of alkali (Table 8), but it was not more than the effect of the alkali itself (see Group 13).

Group 10 (miscellaneous N). Formamide was somewhat effective. Aniline yielded Laccaria proxima, a ureophilous

species. If this had been a result of the alkaline reaction of aniline, there should have occurred some ureobiont fungi also (see Group 13). This point will again be discussed later.

Group 11 (N-free compounds). No particular fungus was obtained, except a deuteromycete which occurred on oil plots (see p. 43). This may support the view that the nitrogenous parts of organism body are the problems, when we regard these substances as composing non-nitrogenous parts of organism body. In the starch plot (791), an unexpectedly larger portion of the starch applied was remaining undecomposed even twenty months after the treatment.

Group 12 (N-free compounds). Some of the alcohols seem to be capable of yielding some ureophilous fungi (Pl. 3, A) but not any ureobiont fungi. This point will again be discussed later. Others were not effective. These may support the view that nitrogen is necessary for the occurrence, at least, of ureobiont species.

Group 13 (alkalis). $NaNO_2$ (Group 8), NaOH, and the potassium alkalis yielded many of the urea fungi (Pl. 3, B). Petersen's results with K_2CO_3 (Petersen, 1970b; p. 3) may fall in this case. The magnesium- and calcium compounds were scarcely effective: they rather produced pyrophilous fungi (Sagara, 1973). The sodium borate solution (pH 9.18 at 25 C, a buffer solution for pH measurement) caused no change.

The occurrence of urea fungi after the treatment with

the alkalis would be attributed to ammonia that must have been released from the soil. This view may be supported by the following evidences : When the soil sample from the O horizon, equivalent to 20 g dry soil, was treated with 100 ml of 1 N KOH solution, gaseous ammonia was at once distinctly detected with Nessler's reagent; with 3.7 g of $Ca(OH)_2$ suspended in 100 ml water, it was only slightly detected; with 100 ml of 0.01 M sodium borate solution, it was not detected at all. It may be added that the spores of Coprinus phlyctidosporus germinated in ammonia water but not in KOH solutions (see p. 110 , footnote). Alkalis, limes above all, have been known to promote the decomposition of organic matter through microbial activities and the production of ammonia in soil (Alexander, 1961; Mitsui, 1955; cf Part IV). The strong alkalis may kill soil organisms and hydrolize proteins, at least to certain extent. These effects also might have contributed to the occurrence of urea fungi.

The quantity of ammonia released from the soil by the alkali treatment would, however, be smaller than those released from the nitrogenous materials applied. This view may be supported by the followings: (i) Generally*, the fruit bodies obtained on the plots of the alkalis were much poorer in number or size, or in both, than those obtained on the plots of urea or the agents of Groups 1-6; (ii) The fungi which require a higher concentration of urea or ammonia (aqueous) to appear, e.g. Amblyosporium botrytis, Cladorrhinum foecundissimum, and Coprinus neolagopus

---

* Except in Gelatinodiscus sp. and Laccaria proxima.

(unpublished), did not occur on the alkali plots.

At any rate, it may be asserted that a high pH value itself is not effective unless any change in the forms of nitrogen is accompanied.

Group 14 (non-basic K^+ or Na^+). No special fungus was obtained. It would mean that K^+ or Na^+ ions themselves were not necessary for the occurrence of urea fungi in case of the alkali treatment (Group 13).

Group 15 (inorganic acids). No particular fungus was obtained. The acids might kill some soil organisms including fungi. The proteins may be hydrolized by the acids. If there had not been acidic conditions, therefore, some urea fungi might have been able to grow there.

Group 16 (killing agents). As regards alcohols, see Group 12 and below. Bonfire sites yielded three ureophilous species (Pl. 3, C). As was on urea plot, they appeared in (Sagara, 1973) the later stages of succession. This point will again be discussed later. Others did not produce any particular fungus. It seems safe to say at least that killing treatment itself does not yield a full series of urea fungi.

"Partial sterilization" (Russell & Hutchinson, 1909) has been known to induce an increase of ammonia in soil (Suzuki, 1961). In paddy soil chemistry it has been clarified that elevation of soil temperature, mixing soil layers, preliminary drying, and heating (and lime application) accelerate the mineralization of organic nitrogens under flooded conditions and can be a substitute for nitrogenous fertilizers (Mitsui, 1955). Cooke (1958 and personal

communication) considered that the restriction of some fungi to burnt ground was probably attributed to certain unexplained changes in available nitrogen. The killings conducted in the present work (except burning) were probably not accompanied with a significant elevation of pH value (cf Part IV). It is a question what fungi occur when the soil is partially sterilized and an alkaline condition is prepared.

Group 17 (organic solvents). No fungus was obtained (see Group 12 as for alcohols).

From the above results it is clear that the agents which may substitute for urea are confined to the nitrogenous materials which will at least eventually release ammonia and cause alkaline conditions and that aqueous ammonia is the simplest of the agents. When the effect of the strong alkalis is taken into account, it may be concluded that a supply of enough NH_4^+ ions together with an alkaline condition is an essential factor required for the sequential occurrence of the urea fungi.

Three ureophilous fungi, i.e. Laccaria proxima, Lactarius chrysorheus, and Rhizopogon rubescens(?), all occurring in the later stages of the fungus succession on the plots treated with ammoniacal materials, draw special attention because they were obtained after the treatment with some non-ammoniacal agents too (Table 8). Thus:

Laccaria proxima with aniline, ethanol (?), n- and iso-amyl alcohol (Pl. 3, A), alkalis (Pl. 3, B), and burning (Pl. 3, C);

Lactarius chrysorheus with NH_4NO_3 (?), ethanol (?), n-amyl alcohol (?), and burning;

Rhizopogon rubescens(?) with $(NH_4)_2SO_4$ (?), NH_4NO_3 (?), n-butyl alcohol (?), and burning.

Laccaria proxima was most prominent for its abundant occurrence (but not so fat as on the plots of ammonia-releasing materials). Cause of the occurrence of these fungi with these agents is still obscure. The sole aspect probably commonly brought about by the treatments with these agents and the ammoniacal materials may be "partial sterilization" (p. 50). If so, ammonia can not be excluded from the causes for the fungal occurrence. But it is clear that the initial alkalinity (see Part IV) is not necessary for these fungi to appear. This may suggest that the occurrence of some urea fungi (ammonogenous fungi) are not dependent on ammonia or ammonia-releasing agents applied but on certain secondary effects. Decomposition products of the dead bodies of microorganisms which predomianted in the early stages may form part of the secondary effects (cf Collybia cookei on p. 27).

Summary

In the field and laboratory, various agents were applied to the soil of Pinus-Chamaecyparis forest in Kyoto to find out some chemicals or agents which substitute for urea in inducing the occurrence of the urea fungi (Part I) and to

determine the essential factor responsible for it. Aqua ammonia was nearly equivalent to urea and the nitrogenous materials which would release a considerable amounts of ammonia and cause alkaline conditions when decomposed were also as effective as urea. The salts of strong acids and ammonium hydroxide (and L-cystine) were not effective unless any alkali was added. Nitrites, nitrates, carbohydrates, oils, lipoid, carboxylic acids, alcohols (for exceptions see below), phenols, aldehydes, mercaptane, salts of strong acids and inorganic acids, sterilizing treatments (for exceptions see below), and organic solvents did not produce any special fungus. Alkalis yielded some of the urea fungi, though their fruiting structures were generally poor. This would be attributed to the liberation of ammonia from the soil. It seems that the supply of enough NH_4^+ ions together with an alkaline condition is essential to cause the occurrence of urea fungi.

Some of the nitrogenous materials more complex than urea or ammonia in their chemical structures brought about the occurrence of some species which were not obtained with urea or aqua ammonia. They are integrated with the urea fungi and discussed together under the general term "ammonogenous fungi", since the non-ammoniacal materials did not produce them.

Aniline, amyl alcohol, and burning yielded some fungi (ureophilous species) which occurred in the later stages of the fungus successions after the application of ammonia -releasing materials or alkalis. This may suggest that

some secondary effects are involved in the fungus successions in question.

Part III

NATURAL HABITATS OF THE AMMONOGENOUS FUNGI

Introduction

To elucidate an ecological or physiological meaning of the fungal occurrence after the treatment of soil with ammonia-releasing agents (Parts I, II), the habitats in nature of the ammonogenous fungi (p. 46) were searched for.

In cases the identifications have been settled, the habitats of the fungi can be known also from taxonomic literature. These will be cited in Part V.

Methods

As expected from the results in Parts I and II, excretions or dead bodies of some terrestrial animals, for instance, may accomplish the changes in soil conditions and fungus flora similar to those observed after the treatment with ammoniacal agents. The spots in nature where such matters were added by chance were examined repeatedly and

the fungi occurred there were recorded. The spots examined are as follows:

a) 21 spots where urine of man was dropped;

b) 14 spots where feces of man (omnivorous) were dropped and decomposed;

c) 2 spots where dead body of cat decomposed;

d) 5 spots where dead body of dog decomposed;

e) 5 spots where body of man decomposed after suicide;

f) 1 spot where night soil was illegally dumped.

These were distributed in various forests of Kyoto and Shiga.

In addition, dungs of wild boar (Sus scrofa leucomystax*, omnivorous) were examined, three samples in the laboratory and one in situ (the Pinus-Chamaecyparis forest, St. 32).

Results and Discussion

The results are shown in Table 10. It should be emphasized that, usually, the fungi in question appeared not on the feces or the dead bodies themselves but on the soils after they were decomposed, leaving bone, scale, or hair (Pl. 2, C, D; see Part IV for the changes in soil properties).

The species which were obtained in the present investigation but not after the treatment with urea or other ammonia-releasing materials (Groups 1-6 in Part II) are:

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* Prof. M. Asahi, Hyôgo Medical College, was kind enough to identify mammalian dungs. To him I am very grateful.

Table 10. The fungi formed reproductive structures exclusively or relatively luxuriantly on the forest grounds where some natural matters happened to be placed and decomposed[a]

For explanation of symbols, see Table 8.

Matters	Human urine	Human feces	Cat carcass	Dog carcass	Human corpse	Night soil	Wild boar dung
Zygomycetes							
Mucor sp. no. 4, 5, & 14[b]							+
Rhopalomyces strangulatus	-				+		-
Ascomycetes							
Ascobolus denudatus(?)	+	+	+	+	+		
Ascobolus sp. no. 2		+		+			
Gelatinodiscus sp.	+	+		+	+		-
Humaria velenovskyi						+	-
Peziza sp. no. 1	+	+					+
Basidiomycetes							
Coprinus neolagopus	-		+	+			-
Coprinus stercorarius	-	+					
Hebeloma spoliatum	+		+	+		+	-
Hebeloma vinosophyllum				+		+	-
Laccaria proxima	+	+				+	
Lactarius chrysorheus	+	+	+				
Lepista nuda						+	-
Lyophyllum tylicolor	+	+	+	+			-
Panaeolina rhombisperma	-					+	-

a. In Kyoto and Shiga.

b. Identities between these and those obtained in Part II are not yet clarified.

Lepista nuda (Fr.) Cooke on the night soil-dumped ground; Mucor spp., Ascobolus crenulatus P. Karst.(?), Chaetomium brasiliense Batista & Pontual, Chaetomium murorum Corda, and many unidentified coprophilous(?) fungi on the remnants of boar dungs.

Richardson & Watling (1968) included Lepista nuda in their list of fungi on dung, but they described its habitat as "on compost and well-manured plots" (see Part V). I myself have never heard or seen it growing on dungs. Its character may somewhat be different from the coprophilous fungi occurring on herbivore dungs, and it seems better to include it in the group of ammonogenous fungi. The fungi obtained on the boar dungs, except Peziza sp. no. 1 (and Mucor spp.?), have been known as coprophilous fungi, according to the list by Richardson & Watling (1968), and they were not obtained after the treatment with ammonia or the ammonia-releasing substances (Parts I, II). Therefore, these will not be discussed further in this paper. At any rate, it is clear that some sorts of dungs are the common habitats for some of the ammonogenous fungi and of the coprophilous fungi (see Part V for Ascobolus denudatus(?), Iodophanus carneus, and Coprinus stercoraius). This point will again be discussed in Part V.

It is strange that the human corpse yielded only a few ammonogenous fungi. The reasons may be considered as follows: (i) The quantity of nitrogen released from the corpse was too large for many of the fungi to grow; (ii) The decomposition period was long enough to bring about

the biological or chemical situations similar to those developed under continuous presence or "repeated application" of nitrogen (see Part IV)(iii); Some of the decomposition products disturbed the normal succession. At any rate, such fungus succession on soil after the degradation of dead animal bodies has not been known in forensic medicine, although, according to Tisdale & Nelson (1966), the effect that dead bodies had on increasing the growth of crops was known around 700 B.C. or even earlier in Old Testament.

Urine-put ground has drawn little attention as the habitat of fungi (see Part V). In this work, only the urine of man was studied. Urines of other animals will also be effective though it is very difficult to detect the spots (the litter turns black, so that it is not "impossible"; see Part IV). Even if it is possible, the fruit bodies of the fungi will rarely be obtained unless the animals are large enough to put a enough quantity of urine: With a small amount of urine, the fungi can not fructify on the soil surface or their fruiting structures are too small or scanty to detect.

Dead bodies of wild animals as well would become the habitats of the fungi in question. Actually, however, dead bodies or decomposition residues of larger vertebrates are scarcely encountered in the field of Japan. Hence there seems to be little chance for the fungi to appear in appreciable quantity under natural conditions. This would be one of the reasons why many of the fungi, especially those small in their reproductive structures and appearing in the

early stages of succession, are new species or new to the Japanese flora (see Part V).

How the ammonogenous fungi live on under natural conditions comes into question. Fecal matters and dead bodies of soil animals may be enough for their hyphal growth or at least to maintain themselves. Exudates of plant roots contain amino acids and other nitrogen compounds, and it is possible that the ammonogenous fungi form mycorrhiza or live on in rhizosphere (see Part I). A little data in this respect were obtained in a laboratory experiment: When the fine rootlets of various trees collected from a Pinus densiflora forest were washed with sterilized water and treated with urea, Ascobolus denudatus(?), Peziza sp. no. 1, Gelatinodiscus sp., Fimaria(?) sp. and Lyophyllum tylicolor appeared on the roots. Dead bodies or waste products of other microbes also may become the source of nutrient for these fungi. The ability to develop basidiospores on "mycelial basidia" (Singer, 1962, p. 16) in Lyophyllum tylicolor (Sagara & Hamada, 1965) may have significance under the conditions in which ammonia or ammonia-releasing materials are supplied in very small amounts.

Summary

Place of occurrence of the fungi which were obtained after the treatment of soil with ammonia-releasing agents was searched for in nature. Human urine, human dung, dead

mammalian bodies placed on soil by chance were found to yield them. Excretions of smaller animals, secretions of plant roots, or other sources in soil, which liberate ammoniacal nitrogens in small quantities, may support their hyphal growth or at least their existence.

PART IV

CHANGES IN SOIL PROPERTIES AND OTHER ORGANISMS AFTER THE TREATMENT WITH AMMONIA-RELEASING MATERIALS

Introduction

To find out the characteristics of the places where the urea fungi (p. 23) or the ammonogenous fungi (p. 46, 58) occurred, changes in soil properties and organisms other than fungi were investigated during the course of the studies of Parts I-III.

Methods

Examination of soil properties

Besides the observation with the naked eye, the changes in pH and water content were examined. The soil samples for these measurements were taken from the transition zone clearly recognizable between L and F in the O horizon. The pH value was determined with the suspension of 4 g wet soil in 20 ml distilled water, using Hitachi-Horiba pH Meter

Model M-5. The water content was determined with 10 g wet soil after oven-drying at 105 C for 5 h.

Observation on other organisms

Responses of plant roots were most carefully studied. The roots placed under observation were the terminal rootlets developing laterally and creeping through the surface layers of soil(O or A1 horizon). These would come, at least partly, under the "humus strivers" in the root system schematically represented by Lyr & Hoffman (1967).

Responses of other organisms were recorded only when they were conspicuous.

Results and Discussion

The changes observed after the urea treatment will be described first, since they were most intensively studied and were typical of those observed after the treatment with ammonia-releasing substances.

Changes after the urea treatment

Changes in soil properties. In the initial stage, the O horizon smelled of ammonia and turned black (Pl. 4, A). Its aqueous extract was reddish-brown just like that of compost, whereas that of the control was almost hyaline.

Rise in soil temperature, as is often observed in compost heap, was not recorded after the urea treatment. The smell then turned to that of compost. The color change and the occurrence of the urea fungi were generally confined to within the plot (Pl. 1, A; cf Pl. 1, B and Pl. 3), showing that there was little movement of urea or its successive transformation products. The black color was discernible for two years or more.

The pH value rised to around 9 within a few days, possibly due to ammonia produced by the decomposition of urea, and then lowered slowly, remaining slightly higher than that of the control even two years or more after the treatment (Figs. 3, 4).

The water content also increased, possibly owing to the increase in water-holding capacity of organic matter by enhanced decomposition (Fig. 3; cf Pl. 4, A) and to the increase in fresh weight of microbial population. In the later stages, however, it often decreased lower than that of the control. Breakdown of the organic matter (raw humus) was dramatically stimulated in the early stages, but its rawest parts resistant to decay were left undecomposed. (Cf Sagara & Hamada, 1965, as for the above points.)

Repeated application of urea to the same plot accelerated the rate of lowering in pH value (Fig. 3). The repetition suppressed the occurrence of urea fungi, and, after six or thirteen times treatments, the urea fungi, especially those expected to appear in the early stages (Group I), rarely occurred (Table 11). The same effect was

(Continued on p. 68)

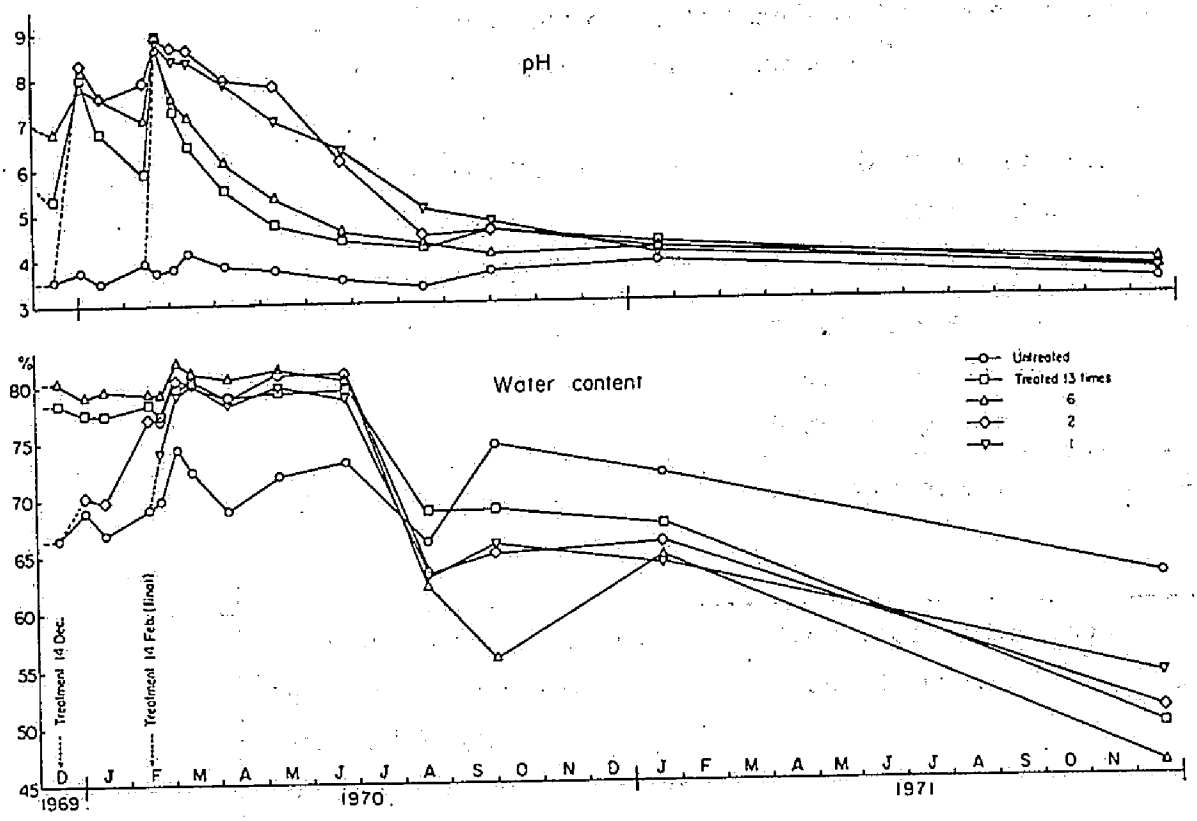

Fig. 3. Changes in pH value and water content in the O horizon of Pinus-Chamaecyparis forest in Kyoto (St. 32, p. 10) after repeated or a single application of 200 g urea-N to 0.5 x 10 m plot. —□— After monthly applications from Jan. 1969 to Feb. 1970 except Jan. 1970. —△— After monthly applications from Aug. 1969 to Feb. 1970 except Jan. 1970. —◇— After double applications, one in Dec. 1969 and another in Feb. 1970. —▽— After a single application in Feb. 1970. —○— Control (untreated). Means of three samples from each plots.

Table 11. Occurrence of fungi after repeated or a single application of urea to soil in the field experiment[a]

Number of fruit bodies appeared are presented roughly by +-+++ in Group I and by the total number in Group II[b].

Number of times of treatment[c]	Occurrence of the fungi of Group I		Occurrence of the fungi of Group II	
13 (Jan. 1969[d] -Feb. 1970)	None		*Hebeloma radicosum*	1
			Hebeloma spoliatum	5
			Laccaria proxima	79
6 (Aug. 1969[d] -Feb. 1970)	*Coprinus* sp. no. 2	+	*Laccaria proxima*	4
2 (Dec. 1969, Feb. 1970)[e]	*Ascobolus denudatus*(?)	+	*Lyophyllum gibberosum*	409
	Lyophyllum tylicolor	++	*Laccaria proxima*	1
	Fimaria(?) sp.	++		
	Coprinus sp. no. 2	+++		
	Peziza sp. no. 1	+		
1 (Feb. 1970)	*Ascobolus denudatus*(?)	+	*Lyophyllum* gibberosum	193
	Fimaria(?) sp.	+	*Laccaria proxima*	1
	Lyophyllum tylicolor	+		
	Peziza sp. no. 1	+		
	Coprinus sp. no. 2	+		

a. In the *Pinus-Chamaecyparis* forest in Kyoto (St. 32, p. 10).

b. See Part I for the grouping of urea fungi.

c. In a single treatment, 200 g N was applied to 0.5 x 10 m plot.

d. Monthly except Jan. 1970. Final application on 14 Feb. 1970.

e. Probably almost equivalent to a single treatment of 400 g N/0.5 x 10 m, since the days between these two applications were so dry and cold that the biological processes were considered hardly to proceed.

Table 12. Occurrence of fungi after a single and double applications of urea to soil[a] in the laboratory experiment

Number of fruit bodies appeared are presented roughly by +-+++ in Ascomycetes and by the total number in Basidiomycetes.

Pot no.	First treatment[b]: mg N /1 g soil[d]	Occurrence after the first treatment		Second treatment[c]: mg N/1 ml /1 g soil[d]	Occurrence after the second treatment	
1	2.5	None		10	*Ascobolus denudatus*(?)	+
					Lyophyllum tylicolor	7
					Coprinus sp. no. 2	5
2	5	*Gelatinodiscus* sp.	+++	10	*Ascobolus denudatus*(?)	+
					Lyophyllum tylicolor	3
3	10	*Ascobolus denudatus*(?)	+	10	*Ascobolus denudatus*(?)	+
		Gelatinodiscus sp.	+++			
		Lyophyllum tylicolor	5			
4	20	*Ascobolus denudatus*(?)	+++	10	None	
		Lyophyllum tylicolor	8			
		Gelatinodiscus sp.	+			
5	40	*Ascobolus denudatus*(?)	+++	10	None	
		Gelatinodiscus sp.	+++			

a. Collected from the O horizon of the *Pinus-Chamaecyparis* forest in Kyoto (St. 32, p. 10).
b. Conducted as Exp. 9c in Tables 6 and 7. c. Conducted as a part of Exp. 9' in Table 7: ←When the fungal occurrence after the first treatment ceased or almost ceased, this treatment ←was added to the same soils. d. In dry weight; see the Methods of Part I.

(Continued from p. 64)

observed in a laboratory experiment too (Table 12). A large amount of urea like 160 or 320 g N/0.5 x 1 m (equivalent to 1600 or 3200 g N/0.5 x 10 m, respectively) did not exert such a effect so far as it was applied at a time (see Fig. 4 for the case of 160 g N/0.5 x 1 m). Repeated addition of urea seemed to shift the urea-to-fungus reaction system. Decrease in carbon-to-nitrogen ratio (or exhaust of some carbon sources) in soil or prevalence of certain soil organisms adapted to such condition (cf "saturation of soil with nitrifying bacteria", Lees & Quastel, 1946a) might be involved in these consequences. A single treatment, in other words, "a sudden addition" (Hora, 1959), may be essential to cause an ordinary succesion of urea fungi (or ammonogenous fungi).

In case of a single treatment, it is not yet known whether there is any upper limit in amount of urea above which the urea fungi can not appear. A part of them did occur on the plot of 4000 g N/0.5 x 1 m (Plot 776, p. 36). The lowering in pH value on this plot was as slow as the cases in the single treatments shown in Figs. 3 and 4.

Changes in other organisms. Damages to higher plants, particularly to herbs, grasses, or seedlings or younger plants of forest trees, were conspicuous when urea was applied at the rates higher than 80 g N/0.5 x 1 m. The rootlets of forest trees were damaged, but the heavier the damage in the initial period, the more striking the growth of new rootlets in the subsequent period (Pl. 4, B).

In the Pinus thunbergii plantation on the sand dune in Tottori (St. 25, p. 10), the new fine roots developing after the initial damage were often devoid of ectotrophic fungal sheath but were equipped with root hairs (Pl. 4, D). Such naked fine roots could not be found in the untreated place (Pl. 4, C). In the Pinus-Chamaecyparis forest (St. 32), on the other hand, the new fine roots of the pine developing after the treatment were not naked, but the variety of mycorrhizas distinguished by color and form was reduced. (Cf Parts I and III for mycorrhiza formation by the urea fungi.)

A dense colonization of green and blue-green algae was observed on another urea plot in the sand dune pine stand mentioned above. In the Pinus-Chamaecyparis forest, a great increase in bacterial cells and certain changes in the population of soil arthropods were observed by H. Ohara and K. Sawada, respectively (unpublished).

Changes after the treatment with other agents (cf Part II)

Changes in soil properties. a) Groups 1-6 (NH_3-releasing materials). Except L-cystine (Group 5), the agents of these groups brought about fundamentally the same changes in soil properties as urea did. Franz (1956) recognized such changes in O horizon after the treatment with anhydrous ammonia. But he reported disappearance of fungi: He might be correct if he observed only the initial stage. At the L-cystine plot (826), the pH value was 2.45 (one sample) in contrast to 4.0-4.3 at the untreated places (24 Oct. 1972) (this compound did not yield any fungus). After the

treatment with calcium cyanamide, the water content of soil was lower than the control even in the early stages, possibly owing to its lime or excessive carbon component, or both (the fertilizer-grade calcium cyanamide contains excessive carbon).

b) Groups 7-9 (non-basic NH_4^+-N, NO_2^--N, NO_3^--N). Except $NaNO_2$ of Group 8 (see Group 13), the agents of these groups did not induce the color change and the dramatic breakdown of organic matter, the first symptoms for the occurrence of the fungi in question. After the treatment with NH_4Cl (Group 7) and $NaNO_3$ (Group 9), the pH value slightly rised but it was still acidic (Fig. 4). The water content also increased but it did not reach the level attained by urea treatment (Fig. 4). The reason for these increases has not been studied.

c) Group 10 (miscellaneous N). Hydrazine hydrate and the amides slightly changed the color to black. Aniline brought about the black color and considerably promoted the organic matter decomposition (and probably the increase in water content). Others did not cause these changes.

d) Groups 11 and 12 (N-free compounds). All the agents of these groups did not cause the above-mentioned color change and breakdown of organic matter. Alcohols seemed to induce an increase in water content. With oils, lipoid, phenol, and cresol, the O horizon turned blackish. But this change was different from that with ammonia-releasing agents (Groups 1-6): The litter appeared as if it had been soaked in oil. With some carboxylic acids, the litter was considerably decolored.

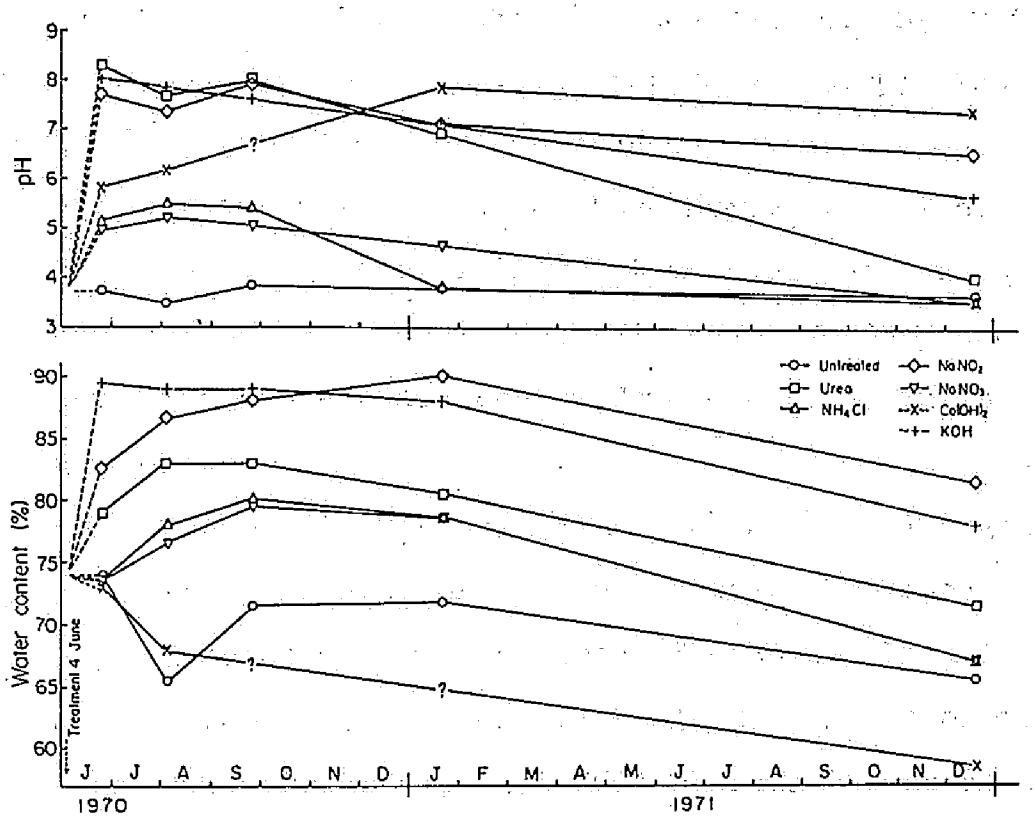

Fig. 4. Changes in pH value and water content in the O horizon of Pinus-Chamaecyparis forest in Kyoto (St. 32, p. 10) after the treatment of 0.5 x 1 m plot with urea or some other agents (see Part II). —□— 160 g urea-N (Plot 711). —△— 160 g NH_4Cl-N (Plot 714). —◇— 160 g $NaNO_2$-N (Plot 717). —▽— 160 g $NaNO_3$-N (Plot 720). —X— 1 kg $Ca(OH)_2$ (Plot 728). —+— 500 g KOH (Plot 729). —○— Control (untreated). Single samples from each plots.

e) Group 13 (alkalis). $NaNO_2$ (Group 8), NaOH, and KOH exerted strong influences on the soil properties as well as urea (Fig. 4; cf p. 49). Their ability to decompose organic matter might be stronger than urea's. Petersen (1970b) also observed "abrupt and thorough changes in the raw humus layer" after the treatment with K_2CO_3 (and Na_2CO_3). Effects of $Ca(OH)_2$ and $CaCO_3$ were similar but much weaker, and these brought about drier conditions (Fig. 4). $Mg(OH)_2$ appeared to be ranked between KOH (or NaOH) and $Ca(OH)_2$ in its effect on the soil.

f) Groups 14-17 (non-basic K^+ or Na^+, inorganic acids, killing agents, organic solvents). The above-mentioned color change and enhanced decomposition of organic matter were not seen with all the agents of these groups. HNO_3 (Group 15, Plot 866) changed the color to yellow. H_2SO_4 (Group 15, Plot 867) caused a burn of the surface of O horizon. After burning (Group 16), the organic matter remaining unburnt beneath the layer of ash and charcoal showed similar changes in pH and water content as those observed after the treatment with limes (Group 13).

Changes in other organisms. a) Groups 1-6 (NH_3-releasing materials). The development of new rootlets after the initial damage was as vigorous and luxuriant as that observed after the urea treatment (Pl. 4, B). An exception was calcium cyanamide: the new root growth was suppressed after the treatment with this chemical, probably owing to lime or excessive carbon, or both, contained in it, or to the dry conditions of soil brought about by this

treatment (p. 70). L-cystine remained in the form of crystals for long period (p. 46) and such new root growth as mentioned above was not observed.

b) Group 7 (non-basic NH_4^+-N). The new root growth was fairly good at higher rates. It should be commented that their nutritional values for higher plants do not differ greatly from those of Groups 1-6, nevertheless, their effect on fungi and soil properties acutely differed from that of the latters.

c) Group 8 (NO_2^--N). See Group 13 for $NaNO_2$. Amyl nitrite might slightly stimulate the root growth.

d) Group 9 (NO_3^--N). No particular response of root was found.

e) Group 10 (miscellaneous N). The agents other than urethane, hydrazine hydrate, and nitrobenzen slightly promoted the root growth.

f) Group 13 (alkalis). $NaNO_2$ (Group 8), NaOH, and KOH heavily damaged the root growth, suppressing the subsequent development. Effect of other alkalis on plant root was not conspicuous but it would probably be parallel with the intensities of alkaline reaction of the chemicals (cf p. 72).

g) Groups 11, 12, and 14-17 (N-free compounds, non-basic K^+ or Na^+, inorganic acids, killing agents, organic solvents). The roots were damaged in the initial periods to various degrees depending on the characters of the agents. Some of the agents slightly promoted the root growth in the subsequent period. After burning (Group 16), the growth of

root was considerably good. But the new rootlets under burnt ground were not so fat as those in the plots treated with the agents of Groups 1-6 (such was also the case in the fungal fruit bodies, see Laccaria proxima on p. 52). Sterilizing treatments stimulate the liberation of ammonia in soil (p. 50). Therefore, the "slightly better growth" of rootlets after the treatments with some agents belonging to these groups and some of the Groups 8 and 10 may be attributed to their toxic effects.

Snails often appeared on the plots treated with the agents of Groups 1-6, 13, and on bonfire sites (Group 16). These are the very places where at least some of the ammonogenous fungi or fireplace fungi occurred. They possibly migrated there to eat hyphae or other microorganisms growing. Some special species of moss colonized commonly on the plots of calcium cyanamide or limes and on the burnt grounds, as was reported by Petersen (1970a, b). The results concerning this respect will be presented in a separate paper.

Changes in the natural habitats (cf Part III)

Changes in soil properties. The changes were similar to those on the plots treated with urea or other ammonia -releasing substances (see p. 63, 69). Some data are shown in Table 13.

Changes in other organisms. The damages to higher plants and the subsequent root development were similar to

Table 13. pH values and water contents recorded with the forest soils (O horizon) on which some natural matters happened to be placed and decomposed[a]

Matters	Date of placement	Date of measurement	pH		Water content	
			The spot	Outside	The spot	Outside
Human urine	16. iv. 66	23. v. 66	7.0	3-4[b]	—	—
Human urine	23. iv. 66	23. v. 66	7.3	3-4[b]	—	—
Cat carcass	ii? 66	6. vi. 66	7.3	3-4[b]	—	—
Human corpse	12? viii. 68	17. ix. 68	5.3	3.6	78.8	72.5
		17. x. 68	3.9	3.5	80.0	70.0
		7. viii. 72	3.7	3.6	50.0	54.0
Human corpse	iv? 68	17. x. 68	5.9	3.2	76.3	70.0
		7. viii. 72	3.5	3.5	41.5	45.0
Human corpse	ix? 68	17. x. 68	7.0	4.6	81.3	61.3

a. In Kyoto and Shiga.

b. Estimated.

those observed after the treatment with urea or other ammonia-releasing substances (see p. 68, 72). This was true even on the spots of human corpse where only a few ammonogenous fungi were obtained (p. 58).

Summary

Changes in soil properties and organisms other than fungi after the treatments employed in Parts I-III were studied to characterize the habitat of the fungi in question (ammonogenous fungi). Color change to black, alkaline condition, higher water content, enhanced decomposition of organic matter, and smell of compost, all occurring in the initial or early stages, and stimulated root growth, occurring in the subsequent stages, were the features commonly found in the O or A1 horizon after the treatments with ammonia-releasing materials which yielded the ammonogenous fungi.

Repetition of the urea treatment to the same soil accelerated the lowering of pH value after the initial rise and suppressed the occurrence of the urea fungi, at least those expected to appear in the early stages: A single treatment may offer the key to induce the normal succession of the ammonogenous fungi.

PART V

TAXONOMIC POSITIONS AND KNOWN HABITATS OF THE AMMONOGENOUS FUNGI

Introduction

A large number of fungus species were obtained on the soils after the treatment with urea, ammonia (aqueous), or the nitrogenous materials which liberate ammonia and cause alkaline conditions (Parts I-III). They were termed "urea fungi" (p. 23) or "ammonogenous fungi" (P. 46, 58). In the present study, taxonomic positions of these fungi were examined and their habitats were, if identified, cited from the literature concerned, to discuss the new findings taxonomically or ecologically.

Methods

The species, including the "doubtful species" (p. 24), are enumerated under the Classes Zygomycetes, Deuteromycetes, Ascomycetes, and Basidiomycetes. Within each Class, they

are arranged in alphabetical order.

In cases the taxonomic status are uncertain, they are morphologically described or taxonomically discussed. Colors of the reproductive structures are usually described after the designations by Ridgeway (1912) and in these cases the color terms begin with capital letters. In cases identifications have been settled or approximated, the habitats mentioned in the taxonomic literature, on which the identifications are based, are listed up.

The specimens are preserved in my personal herbarium.

Enumeration

Zygomycetes

1) Mucor spp.

Identifications are not yet done (cf Tables 8-10). Sp. no. 2 appears to be close to M. hiemalis Wehmer.

2) Rhopalomyces strangulatus Thaxt.

This fungus was reported as new to the Japanese flora by Tubaki (1973) on the basis of the specimens obtained in the present studies.

Deuteromycetes

3) Amblyosporium botrytis Fres.

Habitats: "on decaying basidiomycetes, well-rotted wood and plant debris, bone, dung, and isolated from

heathland soil" (Pirozynski, 1969).

4) Cladorrhinum foecundissimum Sacc. & March.

Habitats: on boar dung and isolated from soil and textile samples buried in soil (von Arx & Gams, 1967).

5) Doratomyces putredinis (Corda) Morton & Smith

Habitats: "laboratory contaminant", "on decayed onions" (Morton & Smith, 1963).

6) Oidiodendron truncatum Barron

Habitats: "isolated from soil of mixed wood and ceder bog" (Barron, 1962); isolated from mountain soil (Tokumasu, 1973).

7) Penicillium lividum Westling

Habitats: "isolated from soil, stored cereal products, and other organic materials subject to air or soil borne contamination" (Raper & Thom, 1949).

8) Stysanus medius Sacc.

Habitats: "isolated from soil" (Gilman, 1957); "on dung" (Tubaki, 1954) (of hare, according to personal communication).

Ascomycetes

9) Ascobolus denudatus Fr.(?)

Apothecia sessile or with a short stalk, up to 5 mm diam, yellowish-green. Excipulum covered with groups of subglobular cells with yellowish or almost hyaline walls. Asci 170-270 x 17.5-20 (-22.5) µm. Ascospores 15-17.5 x 7.5-9 µm, when swollen reaching 20-21 x 11-12.5 µm, ornamented with longitudinal subparallel ridges that only

occasionally anastomose.

The specimens referred to Prof. R. P. Korf, Cornell University, were identified as A. denudatus and reported as new to the Japanese flora (Korf, 1965). I am at present not definite on the identity because of the following problems. The ascospores of A. denudatus are (16-)18-22 (-23) x (8.5)9.5-11.5 μm (van Brummelen, 1967) or 17-20 x 8-9 μm (Dennis, 1968). Namely, they are slightly larger than those of the present materials. Cells of excipular warts are rust-brown in A. denudatus according to van Brummelen, whereas in the present materials they are yellowish or almost hyaline. And I have never seen that sort of "unusual" ornamentations as illustrated by van Brummelen.

Petersen (1970b) obtained "Ascobolus denudatus" on the plots treated with K_2CO_3. This may support the identification by Prof. Korf, since the species in question was obtained after the treatment with KOH and other alkalis in the present experiments (Part II).

Habitats of A. denudatus: "on rotten wood and branches, rotten straw and leaves, composted bracken, humid soil, manure pile, tan refuse, honey comb of wasp nest, old carpet, rarely on dung" (van Brummelen, 1967).

10) Ascobolus sp. no. 2 (Fig. 5, Pl. 5)

Apothecia cup-shaped, disc up to 2 mm diam, concave then flat, yellowish-green, becoming dark violet to almost black at maturity; outer surface yellowish-green to grayish green, finely mealy with groups of subglobular or pear-shaped cells. Asci up to 220 x 16 μm, not blued by iodine.

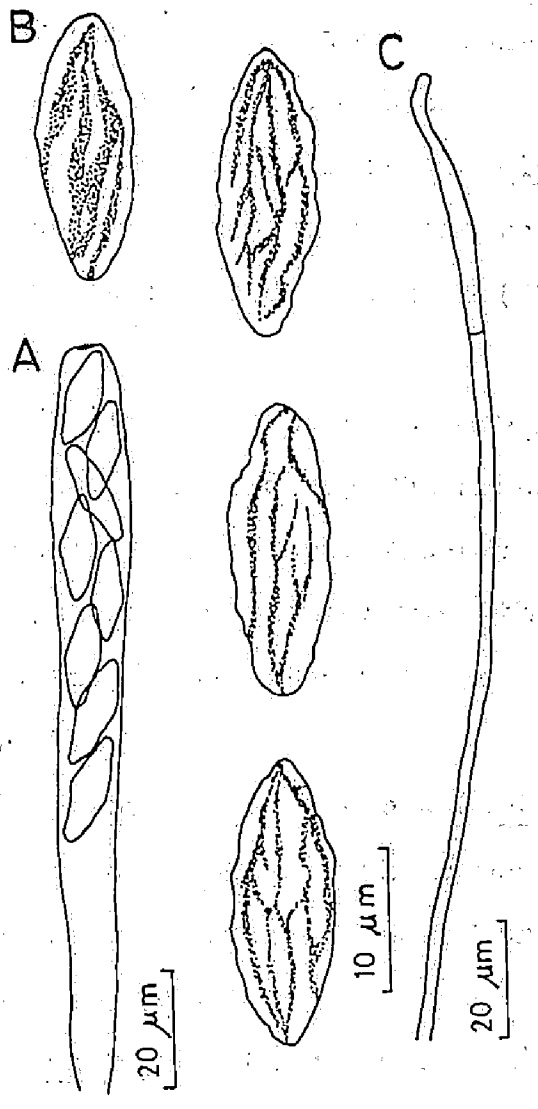

Fig. 5. Ascobolus sp. no. 2. A, ascus; B, ascospores; C, paraphyses.

Ascospores fusiform, 18-21 x 6.5-7.5(-8) μm, when swollen reaching 22.5 x 10 μm, purple then purplish brown, finally almost brown, ornamented with longitudinal thick ridges which can be counted up to several at one side and only occasionally anastomose. Paraphyses simple, almost straight, scarcely enlarged above, 2.5-3 μm thick.

This species appears to be near A. epimyces (Cooke) Seaver, but the spore ornamentaion seems to be somewhat different from those described by Seaver (1942) and van Brummelen (1967):

Seaver stated, "spore-sculpturing consisting of delicate lines (apparently ridges) which anastomose and give the spore a decidedly striate appearance"; Van Brummelen described more abundant and thinner ribs than the present materials and stated, "ornamented with longitudinal anastomosing lines".

Habitats of A. epimyces: "on old Corticium, but apparently on the remains of some slime mould" (Seaver, 1942); "on rotten wood, rotten leaves of trees and old paper" (van Brummelen, 1967).

11) Byssonectria aggregata (Berk. & Br.) Rogerson & Korf

Habitats: "on plant debris on moors and wet ground" (Dennis, 1968, under the name Octospora carbonigena).

12) Chaetomium globosum Kunze ex Fr.

Habitats: isolated from or collected on almost all kinds of substrata including dungs of various animals (Skolko & Groves, 1953; Udagawa, 1960).

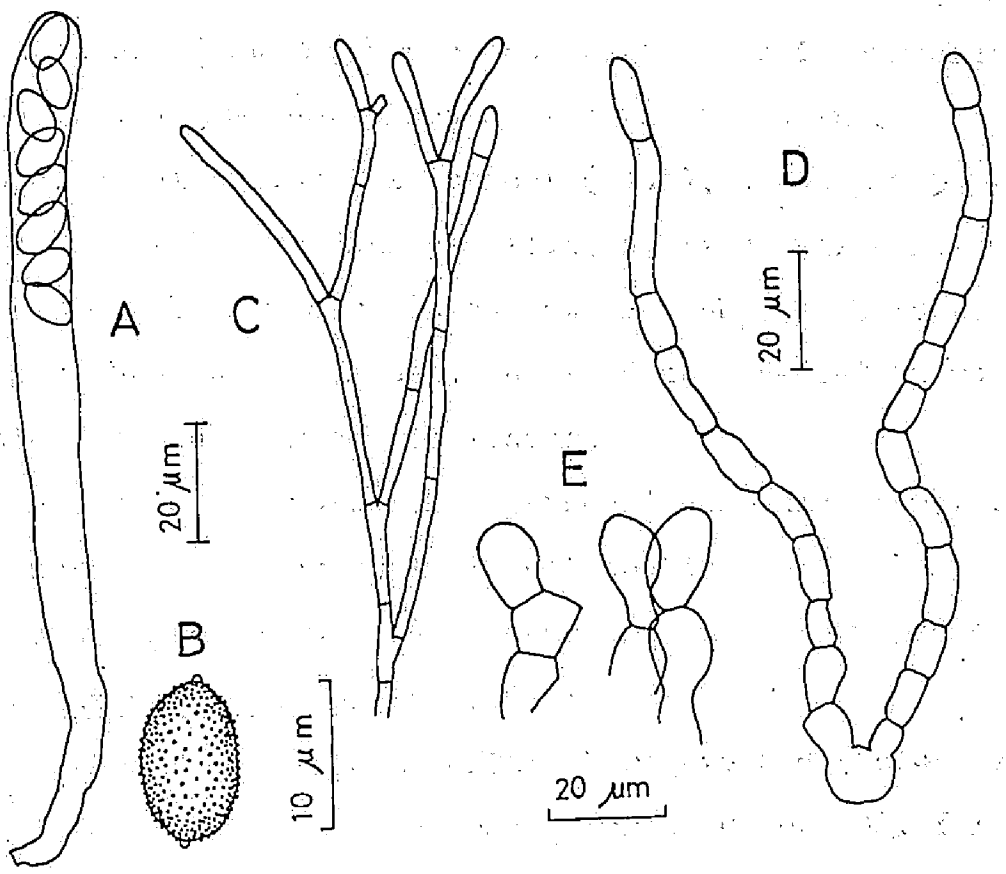

Fig. 6. Fimaria(?) sp. A, ascus; B, ascospores; C, paraphyses; D, hairs of outer surface; E, marginal hairs.

13) Fimaria(?) sp. (Fig. 6; Pl. 6, A)

Apothecia usually less than 6 mm diam, rarely more than 1 cm across, cup-shaped, sessile, disc flat or slightly concave, Orange-Pink, Pale Flesh Color, Pale Salmon Color, or Seashell Pink; outer surface slightly paler, often dotted upwards with brown, obtuse, septate, thin-walled, adpressed hairs which tend to occur in bunches at the margin, giving it a minutely dentate, brown appearance. Asci operculate, 110-160 x 8-12 µm, not blued at the tip by iodine. Ascospores uniseriate, elliptical (oval), 10-12.5 x 5.5-6.5 µm, warted with very fine papillae, hyaline, white in mass, with two guttles in certain cases. Paraphyses slender, straight, branched, scarcely enlarged upwards and up to 2.5 µm thick, septate, green in iodine.

According to Prof. Korf (personal communication), the spore markings do not stain in cotton blue and de Bary bubbles are prominent. This fungus may be a new species.

14) Gelatinodiscus sp. (Fig. 7; Pl. 6, B, C)

Apothecia cup-shaped, often short-stalked (helotioid), expaned to sessile, disc concave, Parrot Green, Oil Yellow, Yellowish Oil Green, Citrine or Dark Citrine, Sulphine Yellow when a little dried, hymenium Citron Yellow to Strontian Yellow when longitudinally sectioned; flesh rather thick; outer surface concolous with some shade of brown, darker towards the base, finely scurfy with groups of thin-walled, globose cells. Asci operculate, 160-200 x 8.5-11 µm, turn blue at the tip (sometimes all over) with iodine. Ascospores uniseriate, oblong-elliptic, 15-17.5

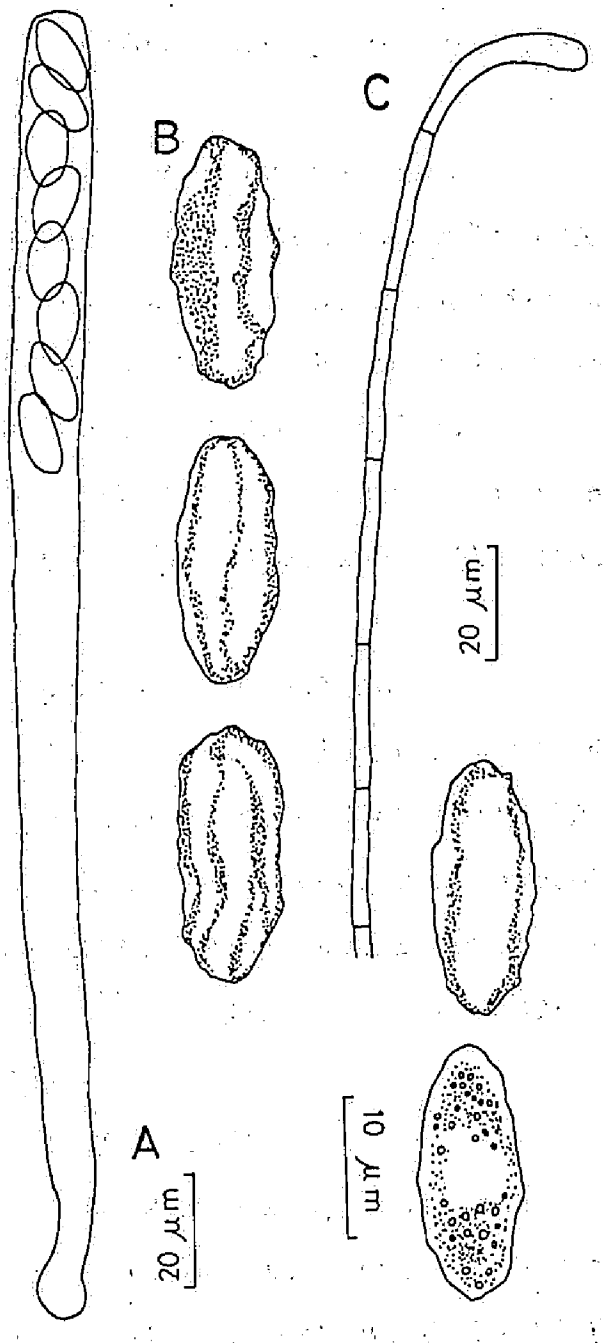

Fig. 7. Gelatinodiscus sp. A, ascus; B, ascospores; C, paraphysis.

x 6-7.5 μm, ornamented with low, irregular ridges. Paraphyses simple or forked at the base, about 2.5 μm wide, curved, slightly clavate and up to 5 μm thick at the tip, septa many.

This fungus may be a new species.

15) Humaria velenovskyi (Vacek in Svrček) Korf & Sagara

This new combination was published together with some descriptions on the basis of the materials obtained in the present studies (Korf & Sagara, 1972).

Habitats: on burn site, humid soil among mosses and conifer needles (Svrček, 1948, under the name Lachnea velenovskyi).

16) Iodophanus carneus (Pers.) Korf

Habitats: "on dung, rotting vegetable matter, including textiles and rope, and on soil" (Dennis, 1968).

This species has been known as a coprophilous fungus (Richardson & Watling, 1968), but recently it was found on burnt ground (Petersen, 1970a) and on $CaCO_3$-treated plots (Petersen, 1970b).

17) Melastiza sp. (Fig. 8).

Apothecia up to 7 mm diam, sessile or very short-stalked under certain conditions, disc concave then flat or convex on aging, Capucine Buff, Salmon Buff, Light Ochraceous -Salmon, Buff Pink or Pinkish Buff; outer surface dotted with brown, obtuse, scarcely thick-walled, septate, adpressed hairs which are wider, shorter and more closely set at the margin, giving it a brown, minutely dentate appearance. Asci 140-230 x 10-11 μm, not blued by iodine.

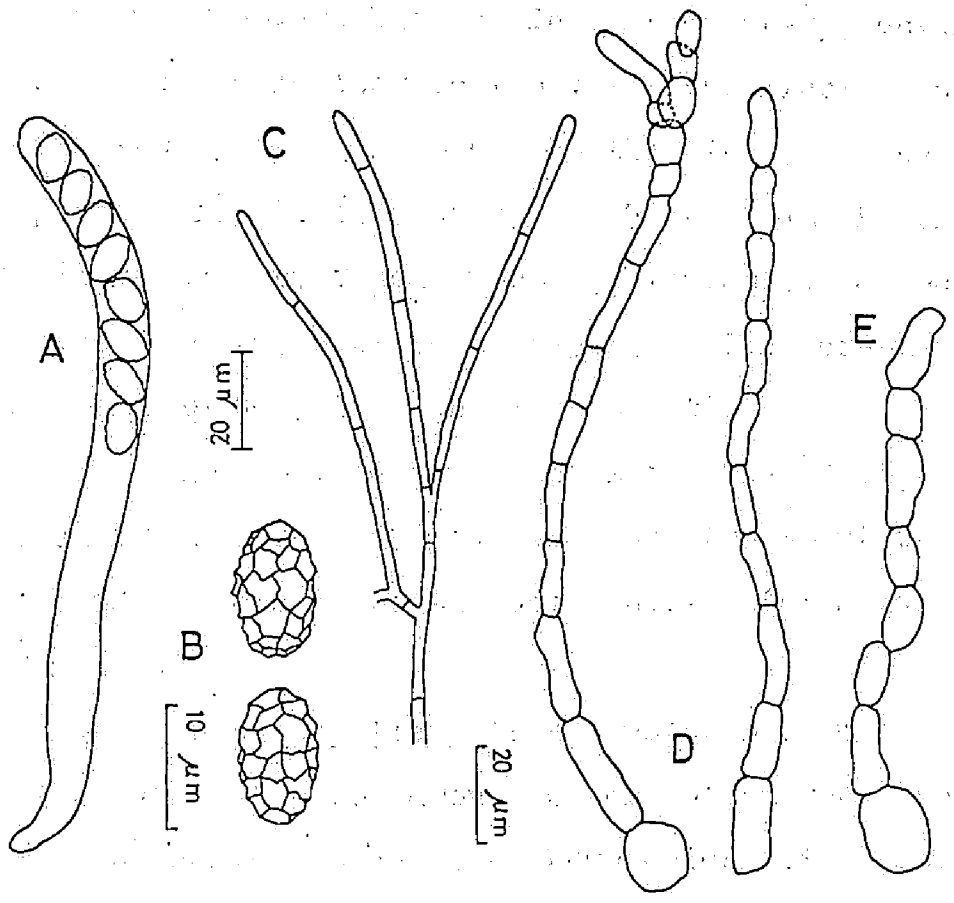

Fig. 8. Melastiza sp. A, ascus; B, ascospores; C, paraphyses; D, hairs of outer surface; E, marginal hair.

This fungus may be near Melastiza flavorubens (Rehm) Pfister & Korf. By the outside view it is difficult to distinguish this species from the Fimaria(?) sp. (p. 84).

18) Peziza sp. no. 1 (Fig. 9; Pl. 6, D)

Apothecia up to 3 cm across, usually less than 1 cm, cup-shaped then expanded, often substipitate, disc almost colorless when young, Pinkish Buff at maturity; outer surface almost colorless to Ivory Yellow, minutely scurfy. Asci 200-230 x 11-12 μm, blued at the tip by iodine. Ascospores elliptical, 12.5-15.0 x 7.5-8.5 μm, hyaline, white in mass, very finely warted, without oil drops. Paraphyses simple, septate, 2.5-3.5 μm wide, distinctly clavate and up to 7.5 μm thick at the tip.

This fungus may be a new species. The "Peziza sp." obtained by Petersen (1970b) after the treatment with K_2CO_3 (and Na_2CO_3) seems to be the same species (cf Sagara, 1973, and Part II).

19) Scutellinia scutellata (Linnaeus ex St. Amans) Lambotte

Habitats: "on wet ground or on sodden wood" (Dennis, 1968); on decaying wood or on humus-rich soil (Imazeki, Hongo & Tubaki, 1970).

20) Trichophaea gregaria (Rehm) Boud.

Habitats: on footpaths in forests of sandy soil, also on burnt ground, etc. (Moser, 1963).

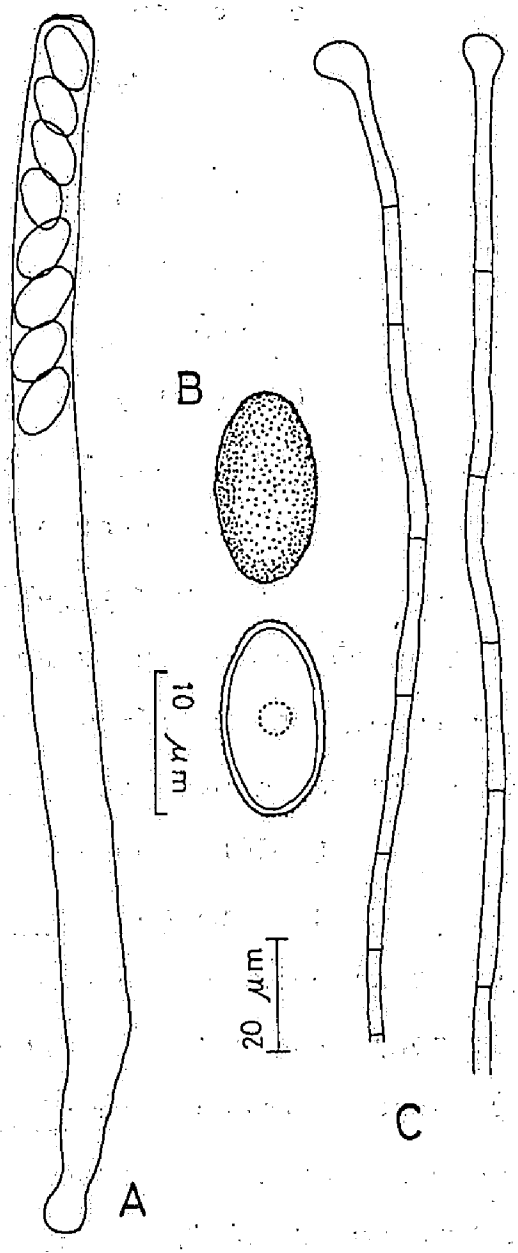

Fig. 9. Peziza sp. no. 1. A, ascus; B, ascospores; C, paraphyses.

Basidiomycetes

21) Cantharellus minor Peck(?)

This may be a color-form of the species.

Habitat of C. minor: "on the ground in woods" (Corner, 1966).

22) Collybia cookei (Bres.) J. D. Arnold

Habitats: on the ground in forests and rotting fruit bodies of fleshy fungi (Imazeki & Hongo, 1965).

23) Collybia(?) sp. (Pl. 7, A)

Pileus strongly pulvinate when young, plane to expanded at maturity, often zonate.

24) Coprinus lagopus (Fr.) Fr.

Habitats: on refuse heap and among leaf litter in forest (Imazeki & Hongo, 1965); on the ground in woods and bushes (Moser, 1967); on refuse heap, arable field rich in organic matter and flower-bed (Aoki, 1970a).

25) Coprinus narcoticus (Fr.) Fr.

Habitats: on decaying wood (Aoki, 1970c); "on decaying straw-mat" (Hongo, 1971); on soil (Moser, 1967).

26) Coprinus neolagopus Hongo & Sagara (1967)

This fungus was reported as a new species on the basis of the specimens obtained in the present studies.

27) Coprinus phlyctidosporus Romagn.

Habitats: on old burn sites (Moser, 1967); on vegetable manure heaps and burnt ground (Imazeki & Hongo, 1965); on vegetable manure heaps and the forest ground (Aoki, 1970b).

28) Coprinus stercorarius Fr.

Habitats: on dung, manured soil, and refuse heap (Imazeki & Hongo, 1965).

29) Coprinus sp. no. 2 (= C. insignis [?] in Sagara, 1973)

This fungus is identical with the one described by Imazeki & Hongo (1965) under the name C. insignis Peck with question mark. They commented that the spores of the Japanese specimens were a little smaller than those of C. insignis and that they applied this name only provisionally. Mr. M. Aoki, Tokorozawa City, and I agree that the carpophores also are considerably smaller than those of C. insignis, which have been said to resemble C. atramentarius (Kühner, 1928; Reid, 1958), and that the "cespitose habit" (Reid, 1958) are not seen in our specimens (personal communication). The "Coprinus sp." obtained after the treatment with K_2CO_3 by Petersen (1970b) seems to be the same species (cf Sagara, 1973, and Part II).

Habitats: on refuse heap (Imazeki & Hongo, 1965); on the forest ground where night soil(?) was dumped (Aoki, 1968a).

30) Coprinus sp. no. 7 (Pl. 7, B, C)

Epicutis of cap slightly green when young, the cells not sphaerical. Stipe not thick at the base, developing down to a long pseudorhiza which does not have a sclerotium. Spores elliptic-navicular, 7.5-11.5 x 3-6 μm, light brown to brown under the microscope.

This fungus may be a new species or at least new to the Japanese flora.

31) Coprinus sp. no. 8 (Pl. 7, D)

Spores elliptic-oval, 8.7-12 x 3-4 μm.

According to Mr. Aoki (personal communication), the shape of the spores is similar to that of C. cortinatus Lange or C. flocculosus Fr. but the position of the germ pore is different from the latters: The pore is apical in C. cortinatus and strongly eccentric in C. flocculosus, whereas in the present materials it is only slightly eccentric. This fungus may be a new species or at least new to the Japanese flora.

32) Hebeloma radicosum (Fr.) Ricken

Habitats: in broad-leaved forest (Moser, 1967); in a close vicinity of stump in broad-leaved forest (Imazeki & Hongo, 1965).

33) Hebeloma spoliatum (Fr.) Karst.

Habitats: in mixed forest (Moser, 1970); in mixed forest of pine and broad-leaved trees (Imazeki & Hongo, 1965).

34) Hebeloma vinosophyllum Hongo

This species appears to be close to H. sarcophyllum Peck which is said to be larger and stouter with different shaped cystidia (Hongo, 1965). Revision of the classification of these two fungi may be necessary after observing the response of the latter to urea treatment: Larger and stouter specimens were obtained after the urea treatment (Parts I, II).

Habitats: "in broad-leaved and conifer forests" (Hongo, 1965); refuse heaps, particularly of garbage of

animal matter (Aoki, 1968a).

35) Laccaria proxima (Boud.) Pat.

Habitats: on the ground or among the sphagna in forests (Imazeki & Hongo, 1965); in moor, often among Sphagnum (Moser, 1967).

This fungus was found to be a fireplace species (Sagara, 1973; Part II). On burnt ground, Moser (1949) collected Laccaria laccata (Scop.) Berk. var. rosella (Batsch) Sing. and Petersen (1970a) collected L. laccata, L. proxima, and L. tortilis ([Bolt.] S. F. Gray) Cooke. They did not, however, come to recognize that these Laccarias prefer burnt ground, excepting that Moser (1949) mentioned in a footnote that L. laccata var. rosella would probably be placed under the group "anthracophilous fungi". Franz & Laub (1959) obtained L. amethystina on limed plot. All these species are so similar to each others in the morphological characters that the identities should be revised, if possible, on the basis of world-wide data of the experiments similar to those mentioned in Parts I and II.

36) Lactarius chrysorheus Fr.

Habitats: on the ground in forests (Imazeki & Hongo, 1957); in broad-leved forest, especially of oaks and chestnuts (Moser, 1967).

This fungus was found to be a fireplace species (Sagara, 1973; Part II). It may be mentioned in this connection that Lohwasser (1953) recorded a luxuriant occurrence of Lactarius deliciosus on limed plot (cf Historicals).

37) Lepista nuda (Fr.) Cooke

Habitats: on the ground in forests and bamboo stands (Imazeki, Hongo & Tubaki, 1970); "on compost and well-manured plots" (Richardson & Watling, 1968).

38) Lepista subnuda Hongo

Habitats: on arable field rich in organic matter (Imazeki & Hongo, 1965).

39) Lyophyllum constrictum (Fr.) Sing. or L. leucocephalum (Fr.) Sing.(?) (Pl. 8)

Carpophores almost pure white all over. Pilei 1.5-5 cm. A couple of stipes often develop from a long psudorhiza. In a pure culture on agar, the stipe developed first and the pileus subsequently, and the pseudorhiza was not formed. Smell strong of meal. Spores echinulate, elliptic-oval (in dried specimens).

Whether the carpophore has partial veil or not has not been determined. The former species has the veil, but the latter not. On the other hand, the latter forms a long pseudorhiza whereas the former not. Thus, whether this fungus is L. constrictum, L. leucocephalum, or any other species akin to these can not be clarified.

Habitats of L. constrictum: "pastures, especially where the grass is scorched by urine, and amongst short grass under conifers" (Rea, 1922, under the name Lepiota constricta); "grassy pastures, in spaces where the grass is scorched by horse-urine" (Lange, 1935-40, under the name Tricholoma constrictum).

Habitats of L. leucocephalum: "deciduous woods" (Rea,

1922, under the name Tricholoma leucocephalum).

40) Lyophyllum gibberosum (J. Schaeff.) M. Lange

This fungus was reported as new to the Japanese flora by Hongo (1972) on the basis of the specimens obtained in the present studies.

Habitats: on footpath in pine forest, together with Omphalia maura (charcoal remnants no longer detectable) (Schäffer, 1942); on the humid place in coniferous forests, particularly among the mosses which are often demolished by the fungus, but also on the needle litter (Lange, 1954).

Moser (1949) collected this fungus on burnt place, but Lange (1954) and Lange & Sivertsen (1966) denied the occurrence on burnt ground as its general character. On the other hand, it is hard to understand the Lange's observation "mosses being often demolished by the fungus" (see above; translated from his French text): I can not imagine that this fungus demolishes mosses; The death of the mosses might have been caused by some other (ammoniacal?) agents as supposed from the presnet studies (Parts I-IV).

Petersen (1970b) obtained this fungus on the plots treated with K_2CO_3 (cf Sagara, 1973; Part II).

41) Lyophyllum tylicolor (Fr. ex Fr.) Lange & Sivertsen

This fungus was reported as new to the Japanese flora by Hongo & Sagara (1967) under the name L. tesquorum on the basis of the specimens obtained in the present studies.

Habitats: "wood of Picea (boarders of drives, etc.)" (Lange, 1935-40, under the name Collybia erosa); "inside and outside of woods of Fagus, on the ground covered with short

moss" (Lange, 1935-40, under the name Collybia tylicolor); "on what apparently were the very decayed remains of some fleshy fungus" (Smith, 1941, under the name Collybia olympiana): among mosses in the mixed forest of Pinus densiflora and broad-leaved deciduous trees (Aoki, 1970d).

Petersen (1970b) obtained this fungus on the plots treated with K_2CO_3 (cf Sagara, 1973; Part II).

42) Panaeolina rhombisperma Hongo (Fig. 10)

This fungus was described as a new species by Hongo (1973) on the basis of the specimens obtained in the present studies.

43) Panaeolina(?) sp. no. 1 (Fig. 10; Pl. 9, A-C)

This fungus is undistinguishable from Panaeolina rhombisperma by the outside view but is different in the shapes of spore and cystidium. See the next species for further discussions.

44) Panaeolina(?) sp. no. 3 (Fig. 10; Pl. 9, D, E)

This fungus is undistinguishable from the preceding two species by the outside view but is different in the shapes of spores and cystidium. One of the characteristics common in these three species is that the pilei are often covered with drops of water(?). Sp. nos. 1 and 3 may also be new species belonging to Panaeolina or its related genera.

45) Rhizopogon rubescens (Tul.) Tul.(?)

The fruit bodies obtained in the present studies are harder and more elastic with thicker peridia (ca. 500 μm) than the ordinary ones which are popularly collected in the

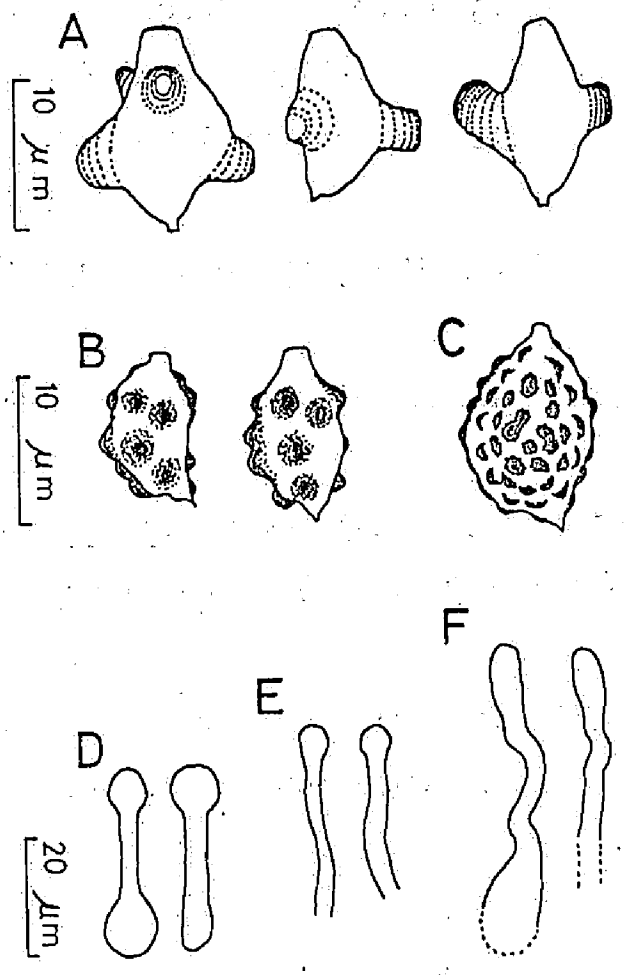

Fig. 10. Panaeolina rhombisperma, Panaeolina(?) sp. no. 1, and Panaeolina(?) sp. no. 3. A-C, basidiospores of respective species in this order. D-F, cheilocystidia of respecitve species in this order.

pine forests along sea coast. The present material may be an upland form of the species. The "Rhizopogon sp." described by Aoki (1972) appears to be the same form.

Habitats of R. rubescens: embedded in the ground of pine forests on coastal sand dunes (Imazeki & Hongo, 1965); on cliffy ground (Aoki, 1972).

This fungus was found to be a fireplace fungus (Sagara, 1973; Part II).

46) Rhodophyllus babingtonii (Blox.) Quél. f. Japonicus Hongo

Habitats: under trees in garden (Imazeki & Hongo, 1965).

47) Rhodophyllus lampropus (Fr. ex Fr.) Quél.

Not the one in Lange (1935-40) nor the one in Kühner & Romagnesi (1953).

Habitats: grassy places (Moser, 1967, under the name R. sodalis); "on soil in deciduous woods" (Hesler, 1967).

Discussion

Among the fungi obtained in the present studies (Parts I-III), the followings were found or are likely to be new species: Ascobolus sp. no. 2, Fimaria(?) sp., Gelatinodiscus sp., Melastiza sp., Peziza sp. no. 1, Coprinus neolagopus, Coprinus sp. nos. 2, 7, and 8, Panaeolina rhombisperma, Panaeolina(?) sp. nos. 1 and 3. And the followings were or will be reported as new to the Japanese flora:

Rhopalomyces strangulatus, Amblyosporium botrytis, Cladorrhinum foecundissimum, Doratomyces putredinis, Penicillium lividum, Ascobolus denudatus(?), Byssonectria aggregata, Humaria velenovskyi, Iodophanus carneus, Trichophaea gregaria, Collybia(?) sp., Lyophyllum constrictum or L. leucocephalum(?), Lyophyllum gibberosum, Lyophyllum tylicolor, Rhodophyllus lampropus. Thus, the present studies are of value in finding new species or latent flora.

As for the following speceis it is hardly possible to relate the results of the chemical treatments (Parts I, II) with the knowledge on their habitats in the literature: Oidiodentron truncatum, Penicillium lividum, Byssonectria aggregata, Humaria velenovskyi, Trichophaea gregaria, Cantharellus minor(?), Hebeloma radicosum, Hebeloma spoliatum, Laccaria proxima, Lactarius chrysorheus, Lyophyllum gibberosum, Rhizopogon rubescens(?), Rhodophyllus lampropus. Thus, the present studies have contributed to unveil latent character of known species.

In some other fungi, it is not impossible to relate the experimental results with the knowledge on their natural habitats in the literature, if we regard the habitats as nitrogen-rich places. The fungi and the habitats with which the experimental results can be convinced are as follows:

Amblyosporium botrytis .... decaying basidiomycetes, bone, dung;

Cladorrhinum foecundissimum boar dung;

Doratomyces putredinis .... decayed onions;

Stysanus medius	dung of hare;
Ascobolus denudatus(?)	manure pile, dung*;
Chaetomium globosum	dungs of various animals;
Collybia cookei	rotting fruit bodies of fleshy fungi;
Coprinus lagopus	refuse heap, arable field rich in organic matter;
Coprinus narcoticus	decaying straw-mat;
Coprinus phlyctidosporus ..	vegetable manure heap;
Coprinus stercorarius	dung, manured soil, refuse heap;
Coprinus sp. no. 2	refuse heap, night soil(?)-dumped ground;
Lepista subnuda	arable field rich in organic matter;
Lyophyllum constrictum(?) .	horse urine-dropped ground*;
Lyophyllum tylicolor	decayed remains of fleshy fungus.

Thus, the present studies have contributed to a deeper understanding of the character of known species.

According to Park (1968), Webster (1970), and Hudson (1972), the term "coprophilous fungi" usually refers to those occurring on herbivore dungs. As seen in the above list, a part of the present group (ammonogenous fungi) have been obtained on "dung". Unfortunately, the sort of dung,

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* This citation is provisional because the identification has not been settled.

i.e. whether of herbivore, of omnivore, or of carnivore, is not mentioned in many literature. This makes it difficult to discuss the relationship between the coprophilous group and the present group: The latter fungi seem to prefer the dungs of omnivores (and carnivores?) which may be richer in nitrogen than the dungs of herbivores (see Part III). At any rate, at least a few species of the present group, e.g. Stysanus medius and Chaetomium globosum, have been known as coprophilous fungi, though, as mentioned later, these two fungi are not principal members of the present group.

Another part of the present group have been collected on burnt ground, i.e. Coprinus phlyctidosporus, Humaria velenovskyi, Iodophanus carneus, and Lyophyllum gibberosum. From the present work (Part II), Laccaria proxima, Lactarius chrysorheus, and Rhizopogon rubescens(?) are added here. Except in Laccaria proxima, the constancy of occurrence on burnt ground seems rather small. At nay rate, the present group and the fireplace group hold some species in common.

It would be the first time that a part of the coprophilous fungi and a part of the fireplace fungi were obtained by the treatment of soil with chemical agents which release ammonia. It is rather strange that the coprophilous fungi have scarcely been related to nitrogen by the previous authors. (The fireplace fungi have been studied in view of ash or dry distillation, cf Historical.)

These points will further be discussed in the General

Discussion.

A larger number of the fungi enumerated above have the spores with rough surface (either with spines, warts, ridges, etc.). To save space the smooth-spored species are listed below.

Mucor spp., Rhopalomyces strangulatus, Cladorrhinum foecundissimum, Doratomyces putredinis, Penicillium lividum, Stysanus medius, Byssonectria aggregata, Chaetomium globosum, Iodophanus carneus, Trichophaea gregaria, Cantharellus minor(?), Collybia cookei, Collybia(?) sp., Coprinus lagopus, Coprinus neolagopus, Coprinus stercorarius, Coprinus sp. nos. 7 and 8, Rhizopogon rubescens(?).

The group of ammonogenous fungi, particularly of urea fungi (or "ammonia fungi", see the General Discussion), seems to be rather well characterized by the predominance of rough-spored species, though Cladorrhinum foecundissimum, Coprinus lagopus, and Coprinus neolagopus of the smooth-spored group are also important for their relatively frequent occurrence. Or it may be considered that the rough-spored species, together with these three smooth-spored species, constitute the principal part of the present group. Any biological interpretation for the above facts is not yet prepared.

All the discomycetes (of Ascomycetes) enumerated above are operculate. Most of the fireplace (pyrophilous) discomycetes are also operculate (Seaver, 1942; Moser, 1949;

Petersen, 1970a; Sagara, 1973). Webster (1970) found few inoperculate discomycetes among the coprophiles and thought it curious. The three places (habitats) exemplified by the occurrence of these three fungus groups may have had similar significance to the evolution of fungi. On the other hand, Seaver (1942) suggested that Ascobolus carbonarius, a pyrophilous discomycete, has been evolved from a coprophilous species. Sagara (1973) drew attention to the correspondence of fungus flora between the fireplace group and the present group. Speciation and habitat segregation in these fungi should be studied with the aid of cyto-genetical or physiological investigations.

Summary

Each of the 47 fungus species occurred on soil afer the treatment with ammonia-releasing materials (Parts I-III) was studied in respect of taxonomy and habitat. 12 of them have turned out or are likely to be new species and 15 to be new to the Japanese flora. As for 13 of the 47 species, their occurrence after the chemical treatments can hardly be explained by the knowledge on their habitats obtained from the literature. As for another 15 species, it is not impossible if we regard their habitats as nitrogen-rich places.

Some species of the present group (ammonogenous fungi) have been known to appear, rarely or often, on dung or on

burnt gound.

A larger number of species of the present group have the spores with rough surface. This may characterize the group morphologically.

All of the dicomycetes are operculate. Evolutional relations among the coprophiles, the pyrophiles, and the present fungi can be suggested.

GENERAL DISCUSSION

The phenomena described in Parts I-IV are summerized in Table 14. Thus delimited were the situations under which the ammonogenous fungi (p. 46, 58) occurred. Alkalinity was necessary as an initial condition to induce the occurrence of the ammonogenous fungi but it was not enough. Presence of a considerable amount of NH_4^+ ions or the materials which release ammonia was necessary. Both conditions are fulfilled if ammonia or some materials which release ammonia as a result of decomposition are added to soil. Application of some alkalis to soil may also satisfy such conditions, because some ammonia is liberated from the soil after this treatment. However, it is hard to imagine that any strong alkali is added to soil under natural conditions. Therefore, the soils to which ammonia-releasing nitrogenous materials are added by chance should be the proper places for these fungi to occur in nature.

The simplest form of the effective nitrogens was ammonia (aqueous) and the most complex was proteins. The fungal species obtained in Parts I-III are summerized in Table 15. Here, ammonia is regarded as essential, and the flora obtained by the ammonia treatment is termed ammonia fungi. The suffixes "-biont" and "-philous" are used in the

(Continued on p. 108)

Table 14. A schematic presentation of the phenomeana observed in the organic layer of soil after the treatment with various agents

Phenomena	Aqua ammonia or materials which release ammonia[a]	Salts of strong acids and NH_4OH[b]	Nitrates	Nitrogen -free materials[c]	Strong alkalis	Killing agents
Color change to black	+	-	-	-	+	-
Alkalization	+	-	-	-	+	-
Increase in water content	+	±	±	±	+	?
Enhanced decomposition of organic matter	+	-	-	-	+	-
Smelling of compost ..	+	-	-	-	±	-
Stimulated root growth	+	+	-	?	-	?
Occurrence of the *ammonogenous fungi*	+	-	-	-	±	-

a. Basic, in its own form or when decomposed to ammonia on or in soil.

b. Non-basic.

c. Carbohydrates, oils, lipoid, carboxylic acids, alcohols, phenols, aldehydes, and mercaptan.

Table 15

Table 15. Compositions of fungal species occurred after the treatment of uncultivated soil with some chemical or natural materials

(In alphabetical order)

Agents	Species
Ammonia (aqueous)	Ammonobiont fungi: *Amblyosporium botrytis*, *Ascobolus denudatus*(?), *Cladorrhinum foecundissimum*, *Coprinus neolagopus*, *Coprinus* sp. no. 2, *Fimaria*(?) sp., *Gelatinodiscus* sp., *Hebeloma radicosum*, *Hebeloma spoliatum*, *Hebeloma vinosophyllum*, *Lyophyllum gibberosum*, *Lyophyllum tylicolor*, *Peziza* sp. no. 1 Ammonophilous fungi: *Laccaria proxima*, *Lactarius chrysorheus*, *Rhizopogon rubescens*(?) ..... Ammonia fungi
Urea	Ureobiont fungi: Ammonobiont fungi + *Collybia*(?) sp., *Coprinus narcoticus*, *Coprinus phlyctidosporus*, *Doratomyces putredinis*, *Panaeolina*(?) sp. no. 1, *Stysanus medius*, (*Ascobolus* sp. no. 2, *Chaetomium globosum*, *Collybia cookei*, *Coprinus lagopus*, *Coprinus stercorarius*, *Coprinus* sp. nos. 7 & 8, *Lyophyllum constrictum* or *L. leucocephalum*(?), *Melastiza* sp. *Oidiodendron truncatum*, *Panaeolina rhombisperma*, *Panneolina*(?) sp. no. 3, *Rhodophyllus babingtonii* f. *japonicus*, *Trichophaea gregaria*)[2] Ureophilous fungi: Ammonophilous fungi + *Cantharellus minor*(?), (*Lepista subnuda*, *Rhodophyllus lampropus*)[2] ::::: Urea fungi
Uric acid	The bulk of ammonia fungi
Hippuric acid ...	A part of ammonia fungi + *Penicillium lividum*
Calcium cyanamide	The bulk of ammonia fungi + *Iodophanus carneus* + many pyrophilous fungi[3]
Ammonium acetate	The bulk of ammonia fungi + *Mucor* sp., *Byssonectria aggregata*
Amino acids[1] ...	The bulk of ammonia fungi
Amines	The bulk of ammonia fungi
Peptone	The bulk of ammonia fungi + *Mucor* sp., *Ascobolus* sp. no. 2, *Humaria velenovskyi*
Proteins[1]	The bulk or a part of ammonia fungi
Animal carcass ..	The bulk or a part of ammonia fungi + *Ascobolus* sp. no. 2, *Mucor* spp., *Rhopalomyces strangulatus*, *Scutellinia scutellata*
Human urine	The bulk of ammonia fungi
Human feces	The bulk of ammonia fungi + *Ascobolus* sp. no. 2, *Coprinus stercorarius*
Wild-boar dung ..	One ammonia fungus (*Peziza* sp. no. 1) + many coprophilous fungi[3]
KOH, NaOH	The bulk of ammonia fungi
$Ca(OH)_2$, $CaCO_3$ ..	A small part of ammonia fungi + many pyrophilous fungi[3]
Burning (bonfire)	Many pyrophilous fungi[3] + A few of ammonia fungi (*Laccaria proxima*, *Lactarius chrysorheus*, *Rhizopogon rubescens*[?])

1) To cause alkaline conditions in soil when decomposed (see Part IV).
2) Obtained from the vegetations other than the *Pinus-Chamaecyparis* forest of Kyoto (St. 32, see Part I).
3) To be excluded from the newly defined group "ammonogenous fungi" because these have not been obtained with the nitrogenous (ammoniacal) materials (see Parts II and III).

(Continued from p. 105)

same senses as explained in Part I. The grouping of urea fungi (Part I) is not abandoned since the experimental data with urea are most abundant, covering almost all over Japan. Some other terms, e.g. carcass fungi and peptone fungi, may also be useful to refer to the species which were obtained only after the treatment with such complex materials.

The "fireplace fungi" (Petersen, 1970[a]) and the "coprophilous fungi" (Webster, 1970) are the habitat groups well known long since. I found that three of the species occurring in the later stages of succession of the ammonogenous fungi could be regarded also as the fireplace fungi (Sagara, 1973; Part II). According to the taxonomic literature, some other species of the present group have sometimes or often been collected on burnt ground or on dung (Part V). These overlaps of flora, though small, would suggest that the same or similar changes take place in the soil of these two or three habitats. This may allow one to insist that these two or three groups should be merged into a comprehensive one, but the followings may oppose this idea.

a) As seen in the recent reviews by Park (1968) and Hudson (1972), it has never been recognized that urine or dead animal bodies (except the durable parts, such as hair, feathers, fur, and bone) yield a particular series of fungi <u>on soil</u> after they are decomposed. Further, the dungs of omnivorous mammals (especially of man), which usually lose their shape rapidly and disappear sooner than the dungs of herbivores, seem to have rather scarcely been the object of

mycologist. It should be stressed that such readily -disappearing materials bring about the occurrence of a special array of fungus species.

The "keratinophilous fungi" (Hudson, 1972) may be the ecological group near to the present one. Cooke (1958) suggested that fungi growing on many types of proteinacious materials should be placed in the group of fungi more or less restricted to keratin. But the flora known as keratinophilous fungi (Dominik & Majchrowicz, 1965, 1970; Majchrowicz & Dominik, 1968, 1969; Hudson, 1972) is almost completely different from the present one. And the present studies (Parts II-V) show that it is better to separate the effect of keratin, a scleroprotein containing a larger quantity of cystine, and that of other readily-decomposing proteins (see p. 43 and 69 for the effects of L-cystine; scleroproteins would not liberate so much ammonia in a short time as to cause alkaline conditions).

b) Alexander (1961) stated, "Without question, the fungi occury a dominant position in proteolysis in certain soils, particularly in acid localities. ..... The microbiology of protein breakdown in soil is inadequately understood". He himself or Dojyô-biseibutsu Kenkyû-kai (1966) named none or few fungi in this connection. The present group of fungi may be the very ones to have been mentioned by these authors, as it is most probable from the results of the present studies (Parts II-V) and from some

preliminary observations* that they take part in the transformations (immobilization or mineralization) of ammonia, proteins (except scleroproteins), or ammoniacal compounds degraded from the proteins or excreted by animals as the final products of nitrogen metabolism. It should be emphasized there do exist some special fungi to be related to the above-mentioned phases of the nitrogen cycle.

The present group is not considered as a physiological group. Some or many of it may not "be produced", in the strict sense, by the ammoniacal materials. (cf p. 52) But it seems reasonable to recognize them as a habitat group for their confined occurrence. It is at least safe to raise them as an experimental-ecological group. That is, the ammonogenous fungi can be defined as the group of fungi which sequentially develop reproductive structures exclusively or relatively luxuriantly on the soil after ammonia or nitrogenous materials which release ammonia and cause alkaline conditions are added suddenly. When the very soil (place) is regarded

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* i) In the test-tube culture on Hamada's medium (tap water 1000 ml, glucose 20 g, dry yeast 5 g, 1.0 N HCl 1.6 ml, agar 20 g), many of the fungi, particularly those appearing in the early stages of the succession, showed striking growth when urea was added.

ii) In Coprinus phlyctidosporus, the basidiospores showed a high rate of germination in ammonia water (initial pH 11.0) but no germination in aqueous solutions of KOH (cf p. 49).

as the habitat of the fungi, the changes accompanying the fungus occurrence (Table 14) can be designated as the characteristics of the habitat. The term "proteophilous fungi" preliminarily used to refer to the same group (Sagara, 1973) is abandoned because it can not express the difference within the effect of proteins (p. 109) and because it can not indicate the ineffectiveness of nitrogen-free compounds deriving from proteins by their degradation (Part II).

One may oppose to assigning some of the above-mentioned species for the present group, saying that he once collected the species on "untreated" or "normal" place. This opposition is of course significant, only if he can prove that the very place had never received any effect equivalent to the application of ammonia or ammonia-releasing substances. The general situation met with is that it is not evidently and exactly known by the outside view whether any spot in the natural surroundings has been "untreated" or "treated". On one occasion, for example, I came across a luxuriant occurrence of Hebeloma vinosophyllum on the normal place in a Castanopsis cuspidata forest, which is situated near human dwellings (St. 30, p.10). Digging up the soil under the fruit bodies, I came to excavate bones of a dog. What we could say confidently is that the place had certainly been treated and that the fungus occurred there reasonably. It is on this basis of constancy in the occurrence of the fungi in question that they can be attributed to the group defined and are interested in from physiological and evolutionary point of view.

CONCLUSIONS

A special assemblage of fungus species sequentially developed reproductive structures exclusively or relatively luxuriantly on the soil of uncultivated land after the single application of ammonia or the nitrogenous materials which release ammonia by their decompostion and cause alkaline conditions. A new grouping can be proposed for these fungi under the general term "ammonogenous fungi". They may be the fungal members engaged in the transformations of ammonia, proteins (except scleroproteins), or ammoniacal compounds produced by proteolysis or by nitrogen metabolism in animals.

SUMMARY

1) Sequential formation of reproductive structures on soil (organic layer) by a special array of fungus species (urea fungi) and some striking changes in soil properties after the application of urea were generally observed with a wide variety of vegetations distributed over Japan. This is the basis to assert that the following results obtained with the forests in Kyoto and its adjacent area will be universal.

2) Ammonia (aqueous) or the nitrogenous materials which release ammonia and cause alkaline conditions were as effective as urea. Strong alkalis were somewhat effective as urea, probably owing to their ability to liberate ammonia from soil. Occurrence of NH_4^+ ions together with an alkaline condition seems to offer the key to cause the above phenomena.

Some of the nitrogenous materials more complex than urea yielded some species additional to the urea fungi and they are discussed together.

3) In nature, these fungi appeared on the grounds in forests where human urine, human feces, or dead mammalian bodies had been placed by chance and decomposed. These matters can be considered as the sources of ammonia.

4) The characteristic features commonly observed in

the soil (O and A1 horizons) to which these ammoniacal materials had been added were color change to black, alkaline condition, higher water content, enhanced decomposition of organic matter, and smell of compost in the initial or early stages, and stimulated root growth in the subsequent stages. Repetition of urea treatment accelerated the rate of lowering of pH value after its initial rise and suppressed the occurrence (vegetative [?] and reproductive growth) of many fungi: a single addition is essential.

5) Among the 47 fungus species obtained after the treatment of soil with the ammoniacal agents, 12 were determined or seem to be new species and 15 new to the Japanese flora. The unidentified fungi were morphologically described. Examination of the taxonomic literature revealed that in 13 of the 28 species so far identified the occurrence after the chemical treatment can not be expected from their natural habitats mentioned in it, and that in the rest of the species it can be if we regard the habitats as nitrogen-rich places. It also clarified that a part of the fungi had been known as the coprophilous fungi or the pyrophilous fungi.

6) Despite these partial overlaps of flora with the habitat groups previously recognized, the present fungi should be raised as a group under the general term ammonogenous fungi for their clearer connections with the transformations of ammonia or ammonia-releasing materials in the nitrogen cycle.

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* May be translated as "Illustrations of Japanese Mushrooms", containing monochromatic drawings and morphological descriptions in Japanese. It has been edited by Mr. Minoru Aoki, Tokorozawa City, and circulated among the members of Nihon Kinoko Dôkôkai, which may be translated as "The Amatuers' Association for Mushroom Research, Japan".

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EXPLANATION OF PLATES 1-9

PLATE 1

A. Occurrence of *Hebeloma vinosophyllum* after the treatment of soil with urea (Plot 611): 160 g N was applied to 0.5 x 1 m in *Quercus glauca* coppice of Ôita (St. 16) on 20 July 1968. Photo. on 7 July 1969. See Part I.

B. Occurrence of *Laccaria proxima* after the treatment of soil with ammonium acetate (Plot 350): 160 g N was applied to 0.5 x 1 m in *Pinus densiflora-Chamaecyparis obtusa* forest of Kyoto (St. 32) on 10 June 1967. Photo. on 6 Oct. 1968. See Parts I (p. 18) and II.

C. Occurrence of *Laccaria proxima* on untreated place in the *Pinus-Chamaecyparis* forest (St. 32) (arrow: outside Plot 350 in B). Photo. on 6 Oct. 1968. See Part I (p. 18).

PLATE 2

A. Occurrence of *Coprinus phlyctidosporus* after the treatment of soil with urea: 20 g (in dry weight) of fallen

leaves of Musa xparadisiaca collected at Sata, Kagoshima (St. 4), was treated with 0.2 g N in glass bottle (7.5 cm diam) and incubated at 10 C on 7 May 1967. Photo. on 16 Oct. 1967. See Part I.

B. Occurrence of Lyophyllum tylicolor after the treatment of soil with urea: 20 g (in dry weight) of litter collected from Pinus pumila thicket at Mt. Hakkôda, Aomori (St. 65), was treated with 0.2 g N in glass bottle (6.5 cm diam) and incubated at 10 C on 12 Oct. 1967. Photo. on 26 Dec. 1967. See Part I.

C. Occurrence of Lyophyllum tylicolor after the addition of sea-fish to soil (Plot 331): 2 kg of saurels were placed on the ground in the Pinus-Chamaecyparis forest of Kyoto (St. 32) on 27 May 1967. Photo. on 6 July 1967. See Part II and Fig. 2.

D. Occurrence of Hebeloma spoliatum after the decomposition of dead animal body: A dog carcass was abandoned on the ground in Quercus serrata-Q. variabilis forest of Shiga (St. 51), probably, in Nov. 1966. Photo. on 26 Oct. 1967. See Part III.

PLATE 3 (See Part II)

Occurrence of Laccaria proxima
after the treatment of soil with some nitrogen-free agents

in the Pinus-Chamaecyparis forest of Kyoto (St. 32)

A. Plot 801: 1 kg of iso-amyl alcohol was applied to 0.5 x 1 m on 16 Feb. 1972. Photo. on 1 Oct. 1973.

B. Plot 849: 1 kg of potassium formate was applied to 0.5 x 1 m on 17 Aug. 1972. Photo. on 1 Oct. 1973.

C. Plot 817: A bonfire was conducted for 2.5 h on 24 Feb. 1972. Photo. on 1 Oct. 1973. The arrows indicate the fruit bodies not well photographed. Note that the fruit bodies appeared circularly on somewhat peripheral zone.

PLATE 4 (see Part IV)

A. A profile of the surface layers of forest ground, showing the change of color to black in the O horizon after the treatment with urea (Plot 462 [not mentioned in the Methods]): 200 g N was applied to 0.5 x 10 m in the Pinus -Chamaecyparis forest of Kyoto (St. 32) on 8 Sep. 1967. Photo. on 15 Nov. 1967. The color change was accompanied by an increase in water content and an alkaline condition.

B. Rootlets of Chamaecyparis obtusa developed in the O horizon after the treatment with aqua ammonia, showing a parallelism of luxuriance to the amount of nitrogen applied. Plots 564-566 (see Part II): 40, 80, and 160 g N was separately applied to 0.5 x 1 m in the Pinus-Chamaecyparis

forest of Kyoto (St. 32) on 20 Jan. 1968. Collection and photo. on 10 Mar. 1969. From left: control, Plot 564 (40 g N), Plot 565 (80 g N), Plot 566 (160 g N). Each block 2 g in fresh weight.

C, D. Formation of fungal sheath-free roots in Pinus thunbergii after the treatment of soil with urea. C, an example of ectomycorrhiza from untreated place. D, a fungal sheath-free root (in the same order of branching as the preceding one) developed after the treatment (Plot 770 [not mentioned in the Methods]): 1.6 kg N was applied to 0.5 x 10 m in P. thunbergii artificial stand on the sand dune of Tottori (St. 25) on 14 July 1971. Collection on 27 Nov. 1971 and photo. on 9 Dec. 1971. The latter is equipped with numerous root hairs, whereas the former not at all.

PLATE 5 (see Part V)

Ascobolus sp. no. 2

A. Asci and paraphyses, unstained water mount.

B. Ascospores, unstained glycerine mount.

C. A group of cells on the outer surface of apothecium, yielding mealy appearance, unstained water mount.

PLATE 6 (see Part V)

A. Fimaria(?) sp. Apothecia.

B, C. Gelatinodiscus sp. B, apothecia. C, outer surface of apothecium, unstained water mount.

D. Peziza sp. no. 1. Apothecia.

PLATE 7 (see Part V)

A. Collybia(?) sp. Basidiocarps.

B, C. Coprinus sp. no. 7. B, young basidiocarps, with long pseudorhiza. C, basidiospores, unstained glycerine mount.

D. Coprinus sp. no. 8. Basidiospores, unstained glycerine mount.

PLATE 8 (see Part V)

Lyophyllum constrictum or L. leucocephalum (?)

A. Young basidiocarps developing from pseudorhiza by branching.

B. A mature basidiocarp (front).

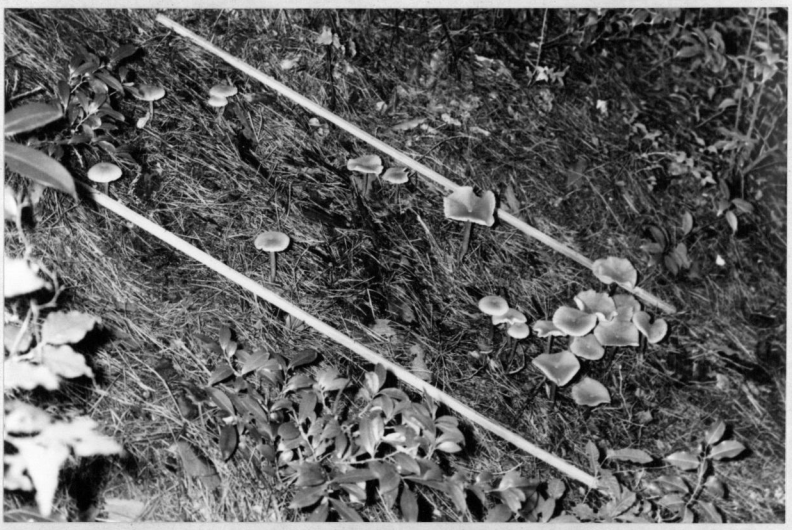
C. Basidiocarps with long pseudorhizas.

PLATE 9 (see Part V)

A-C. Panaeolina(?) sp. no. 1. A, B, basidiocarps. C, basidiospores, unstained glycerine mount.

D, E. Panaeolina(?) sp. no. 3. D, normal (arrow) and abnormal fruiting in culture. E, basidiospores and cheilocystidia, unstained glycerine mount. In the abnormal fruiting (D), the stipe and the pileus were absent or very poorly developed, and only the lamellae were luxuriant.

A



B

C

A

B

C

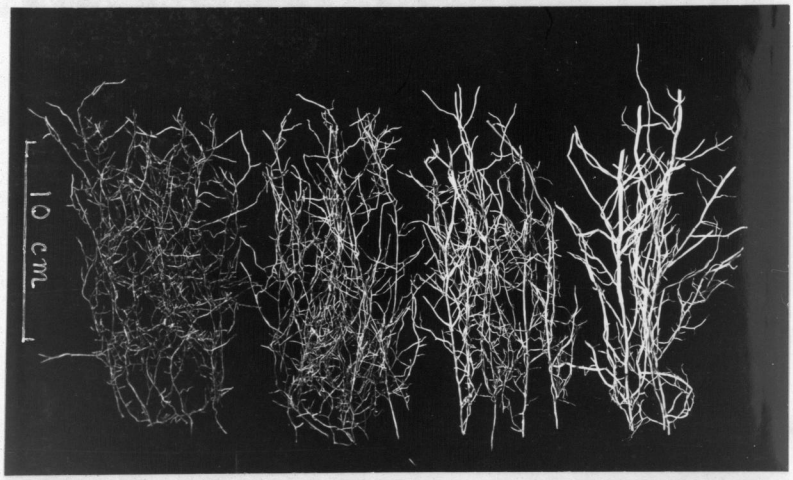
D

A

B

C

A



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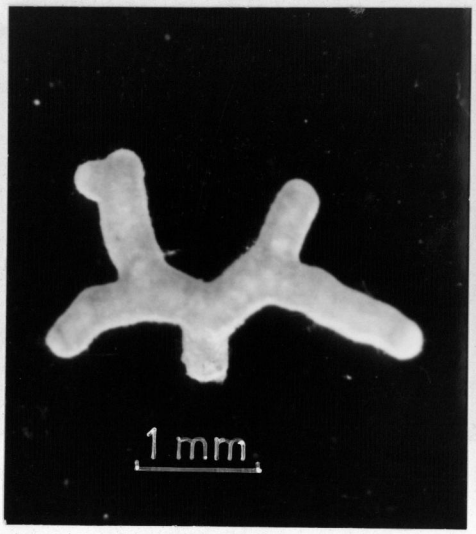

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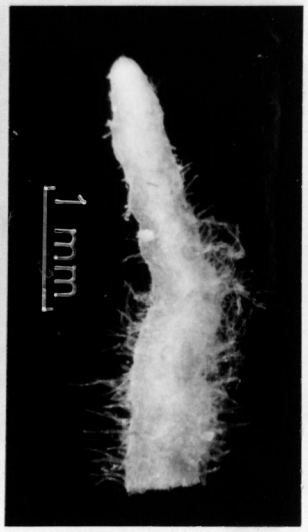
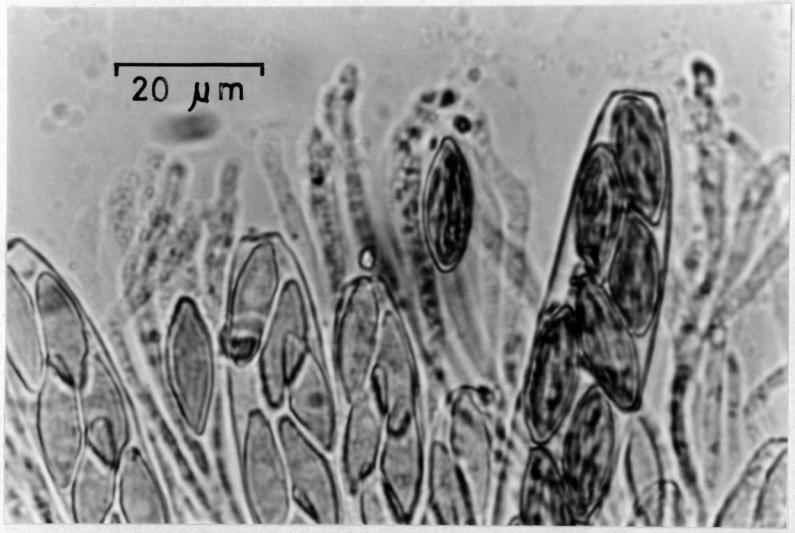


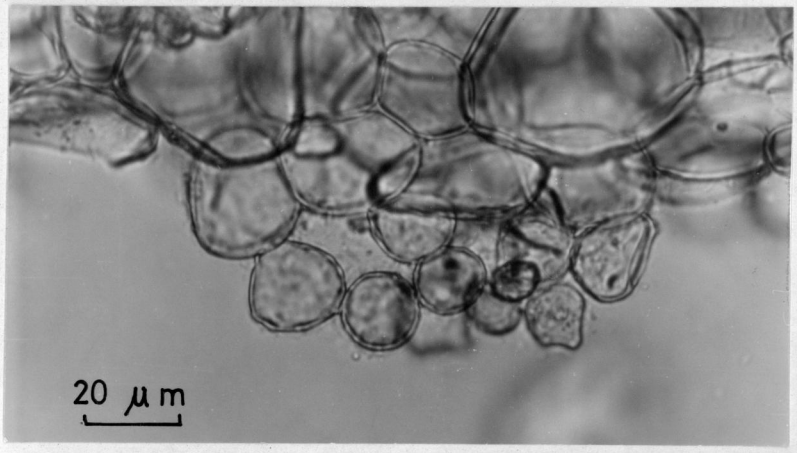
D

Plate 5



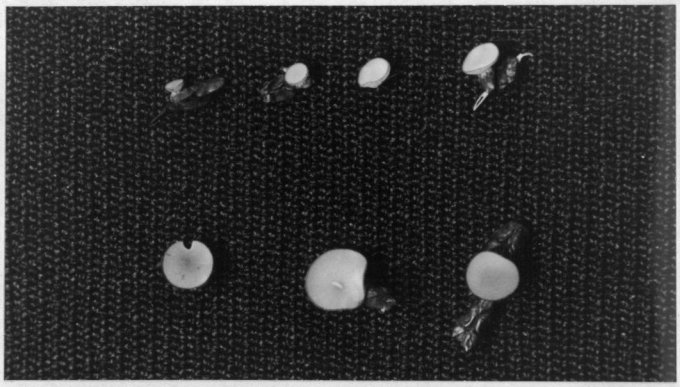
A

B



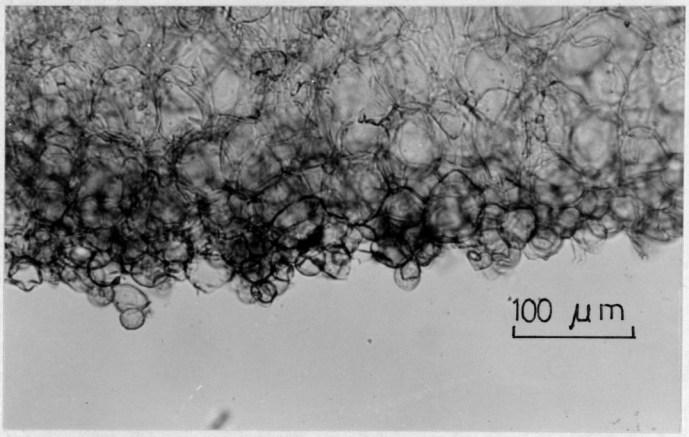
C

Plate 6



A (x 1)

B(x 2)



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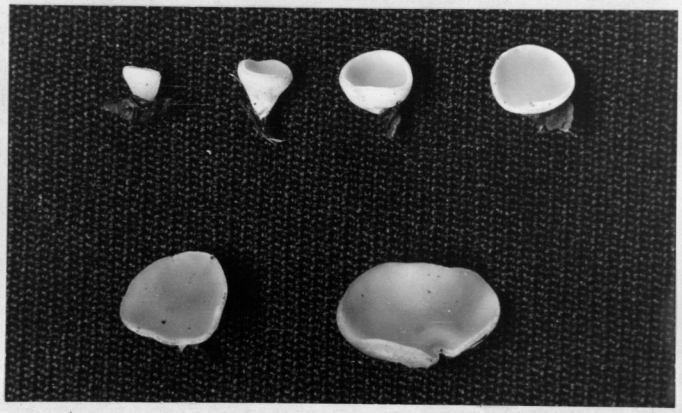
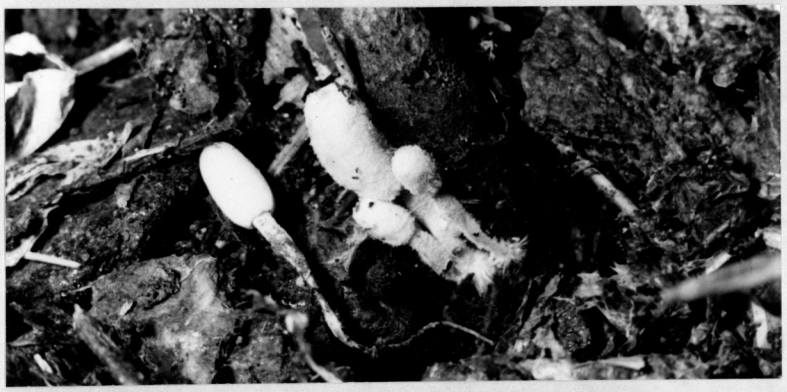


D(x 1)

Plate 7

A (x 2/3)



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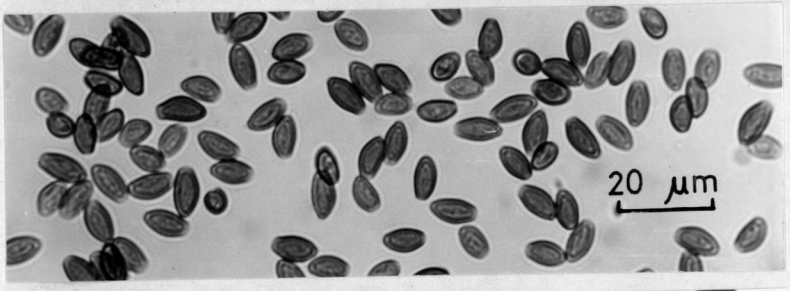

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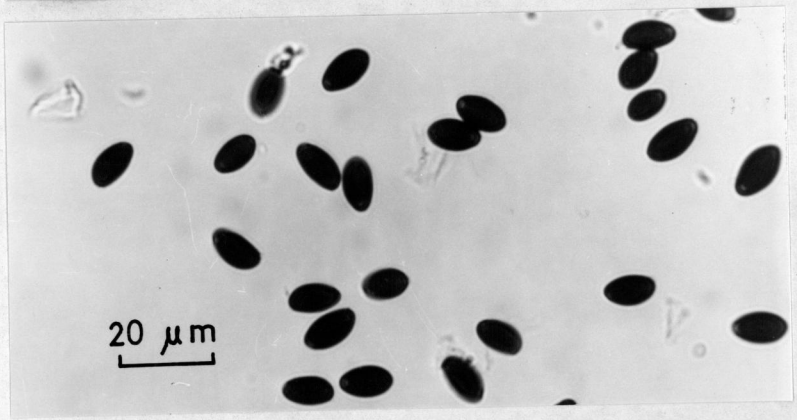


D

A (x 1)

B (x 1)

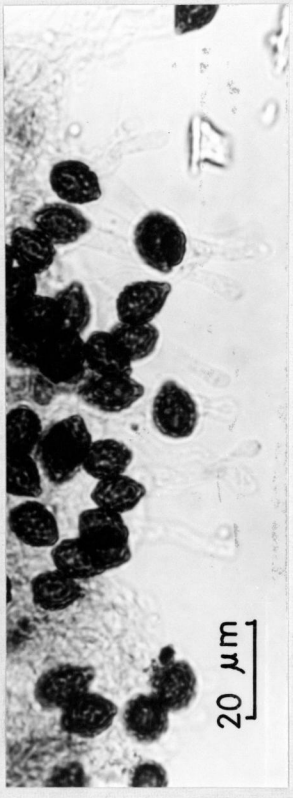
C (x 2/3)

A (x 1.5)

B (x 1.5)

C

D (x 1.7)



E